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SOME RECENT CONTRIBUTIONS BY ENGLISH WORKERS TO THE DEVELOPMENT OF METHODS OF INSECT CONTROL

By C. T. GIMINGHAM, B.Sc., F.I.C.

Ministry of Agriculture, Plant Pathology Laboratory, Harpenden

ADDRESS OF THE PRESIDENT OF THE ASSOCIATION OF APPLIED BIOLOGISTS DELIVERED TO THE ANNUAL GENERAL MEETING ON FRIDAY, 9 FEBRUARY 1940

A LITTLE time ago I came across some extracts from the letters of Sir Joseph Dalton Hooker in which he expresses his feelings on being confronted with the necessity of giving a presidential address. I have no doubt that these letters have been used in this connexion by others in similar circumstances, but one or two sentences from them seem to fit the present situation so precisely that I cannot refrain from quoting them to you. Hooker wrote that he could "see no way to a good address", and, later, "The address is *nowhere* yet and I look on its prospects with a loathing that cannot be uttered." Again he writes: "I do not intend to show any part of the address to my wife, from the conviction that she would burn it all.... I would hiss anybody who would eruct such stuff as I have written under any other circumstances than a presidential martyrdom...." If a man of the distinction and learning of Hooker could write in this strain, my own views on the matter can perhaps be faintly imagined, for added to the normal difficulties is the fact that conditions during the past few months have hardly been conducive to the serious consideration that I should wish to have given to the preparation of a paper to be dignified by the title of "presidential address". I have therefore to ask that you will excuse the rather fragmentary nature of the brief review that follows.

I hope that it may not be without interest and may even serve a useful purpose to recall some instances of work carried out in this country in recent years that has led to definite improvements in practical methods of insect control. A good deal of what I have to say will be familiar to many here, but it is perhaps not a bad thing to look back occasionally and see what general progress can be reported. I am, however, making no attempt at a complete survey which would take much too long, but propose to pick out some examples (mostly dealing with developments in the use of insecticides) having a direct and obvious practical importance. In thus emphasizing the practical aspects of the subject, as seems fitting at a meeting of applied biologists, I do not intend to imply any comparison with investigations of a more academic nature; it is indeed obvious that it is on the basis of work of this latter kind that results having direct application in practice are most frequently obtained.

I propose first to discuss investigations that have been concerned with the control of

APHIDES AND SOME OTHER PESTS OF TOP AND BUSH FRUITS

The use of tar-distillate washes for the control of aphides attacking apple, plum, cherry and bush fruit by winter spraying for destruction of the eggs and for removal of moss and lichen on the bark is so well known and now so routine a process that it is difficult to realize what a menace to successful fruit growing these pests formerly were. Lime washes, caustic-soda washes and spring spraying with nicotine-soap solutions were useless or, at the

best, gave very inadequate control. The discovery of the value of tar-oil preparations for this purpose and of a means of compounding them in a form suitable for use on fruit trees and bushes was not made in this country, but English investigators and English commercial firms have played a prominent part in their development. The first trials were carried out in 1921 and 1922 with a Dutch proprietary product, and entomologists are indebted to a mycologist, Dr Wiltshire, then on the staff of the Long Ashton Research Station, for bringing the original sample from Holland. Similar products were introduced about the same time by commercial firms, and the successful results of a large number of field trials at research stations and by advisory officers and the technical staffs of insecticide manufacturers quickly gave these washes an established position. Many different brands appeared on the market, not all equally efficient, and detailed investigations followed with the object of finding out more about the constituents of these complicated mixtures that are responsible for their insecticidal action and of ascertaining the limitations and extent of their uses. The names of Lees, Tutin, Staniland, Walton, Jary, and Hubert Martin are specially associated with this work.

Among other things, the importance for insecticidal efficiency of the neutral tar oil and the relative unimportance of the tar acids, tar bases, and solid components such as naphthalene and anthracene and, on the other hand, the effect of a proportion of tar acids in facilitating emulsification was shown; and it was also demonstrated that the washes were of considerable value in destroying eggs of the winter moth though not so effective as against eggs of aphides and apple sucker, and that eggs of the red spider were very resistant and little affected. It will be appreciated that results of this kind could not be obtained without a reliable standardized technique for evaluating the insecticidal performance of tar-oil and other egg-killing washes both in the field and under laboratory conditions, and the valuable methods now available are the result of detailed investigations by entomologists at Long Ashton and East Malling. Information of practical value to the fruit grower has been steadily accumulating in the years since the introduction of tar distillate washes, from both chemical and biological points of view, which is leading to a fuller knowledge of their properties and proper uses, and much of this information has been rightly utilized by manufacturers, coordinated with their own investigations and experience and applied in the production of safer and more efficient materials. Much, however, remains to be done, and we are, for example, still without a completely satisfactory explanation of the precise nature of the toxic action upon insect eggs.

It would not be useful to discuss the earlier work here, for it is well known and summaries have been published, but I want shortly to refer to some more recent developments of a different kind which may also have practical results.

Almost all the tar-distillate sprays used are manufactured products, and only in very exceptional circumstances are they prepared from the oils by the user. They are sold in two forms—as miscible oil preparations or as stock emulsions, the so-called Mayonnaise type; both forms need only dilution with water to be ready for use. A proportion of mineral oil is normally added, and various emulsifying agents and other materials are incorporated in the processes of manufacture. The final products are of a highly complex chemical character, and the demand which has arisen for some kind of guarantee that tar-distillate and other oil washes should reach a suitable standard of efficiency and safety in use and be of reasonably constant composition is by no means easy to meet. That a measure of standardization is

desirable in the interests of all concerned is generally agreed, but equally it would certainly be unwise in the present state of our knowledge to attempt to stereotype the composition in any way that would hinder the introduction of improvements that might be proposed as the result of further work. The adoption of agreed minimum specifications for the various types of washes is one method which would go some way towards the end desired, and it was considered that, largely as a result of the investigations to which I have referred, sufficient information as to the relation between composition as revealed by analysis and insecticidal performance was available to justify an attempt to prepare such specifications. Moreover, in 1935 H. Martin of Long Ashton had published an important paper reviewing the accumulated evidence with regard to both tar-oil and petroleum-oil insecticides and suggesting specifications for consideration and discussion. Accordingly, rather more than two years ago, a small Committee was constituted consisting of technical representatives of the Association of British Insecticide Manufacturers, of the Ministry of Agriculture, and of the Research Stations with instructions in the first place to explore the possibility of drawing up a satisfactory specification for tar-distillate washes of the miscible-oil type. No great difficulty was found in reaching agreement on a provisional minimum specification for miscible tar-oil washes, but an essential adjunct to any specification is standard methods of analysis by which it can be checked, and the working out of certain details of the methods has, in this instance, not proved easy. It has involved many meetings and a considerable amount of co-operative analytical investigation by members of the Committee which is still in progress though now nearing completion. In order to make use of the provisional specification, which had been accepted by the Ministry and by the Association of British Insecticide Manufacturers, without waiting for its publication in final form with methods of analysis, the suggestion was made that, as a temporary arrangement, manufacturers of miscible tar-oil washes should give the Ministry a guarantee that their output of these products would conform to the provisional specification. This was agreed to by the Ministry and almost the whole production of these washes in this country has been covered by this guarantee during the spraying seasons 1938–9 and 1939–40. This is of obvious importance to fruit-growers and should greatly facilitate the work of advisory officers in connexion with these washes.

The Committee is also giving attention to the preparation of a similar specification for tar-distillate washes of the stock-emulsion type.

I have specially taken this opportunity of referring to this work on specifications, partly because it is an example of co-operative effort between members of the staffs of commercial firms and of Research Stations and the Ministry which it is hoped will yield results of benefit to growers and manufacturers alike.

Some of the earlier experiments at Long Ashton with preparations of high-boiling neutral tar oils were aimed at control of the apple capsid bug (*Plesiocoris rugicollis* Fall.) and the common green capsid bug (*Lygus pabulinus* L.), which were proving a source of great trouble to growers; and promising results were obtained on apples but not on currants. At about the same time, investigations at the South-eastern Agricultural College at Wye, in which high-boiling petroleum and tar-oil preparations were compared, indicated that the former are more effective than tar oils against the eggs of capsid bugs; and as the result of much further work in which investigators at Long Ashton, Wye, Cambridge and East Malling took part, petroleum-oil washes have come into general use for control of these pests. They have also

proved of value under certain conditions for the destruction of eggs of the fruit tree red spider (*Oligonychus ulmi* Koch), though the results obtained have been less consistent.

Petroleum-oil preparations of this type are, however, of little use for control of aphides and apple sucker, and it was natural that attempts should follow to produce a combined wash capable of dealing in one application with all fruit pests susceptible to treatment by winter spraying. Suitable mixtures of tar and petroleum oils were prepared and tested experimentally at Long Ashton, Wye and Cambridge and have been placed on the market, but, though successful results were obtained under some conditions, difficulties were met with arising partly from risk of bud damage connected with the higher concentration of oils, and such mixed washes appear to have only a limited use.

The problem of finding a thoroughly safe general winter wash, much desired by growers in these days of labour shortage and high costs, has also been approached from another point of view. Investigations on materials toxic to insect eggs and of possible practical value as spray-fluids have not been confined to tar and petroleum oils. In the course of a laboratory survey of the toxicity to aphides and to some other insect eggs of a number of groups of synthetic organic compounds, carried out partly with the object of attempting to correlate toxicity with physical properties or chemical constitution, Tattersfield and his co-workers at Rothamsted found that several of the aromatic nitro compounds were very toxic to insect eggs and that 3:5-dinitro-*o*-cresol was definitely the most active member of this group. This compound and also its sodium salt proved lethal to the eggs of several species of moths, under laboratory conditions, at concentrations as low as 0·1–0·025 %. At the lower concentration, hatching was not always entirely inhibited, but within a few hours of emergence or partial emergence, nearly all larvae died. Arising from these observations, experiments were undertaken during 1925–8 on the possible utilization in practice of di-nitro-cresol as the active constituent of an egg-killing wash for fruit trees. Washes containing di-nitro-cresol at 0·20–0·25 % or the sodium salt at equivalent strength were prepared, and field experiments on plums, apples and currants carried out in co-operation with Massee of East Malling and later with the assistance of several advisers in entomology in various parts of the country. In general, the results of the laboratory work were confirmed. The sprays were almost completely effective against eggs of aphides and apple sucker and greatly reduced winter moths; they had a general cleansing effect on the bark of the trees and, though injurious to foliage, appeared to be safe for use on trees in dormant condition. The effects were indeed similar to those produced by a good tar-distillate wash at the usual strength. A disadvantage in practice was the yellow-staining property of di-nitro-cresol and its salts.

These results, though fairly conclusive, remained unutilized in practice, perhaps partly owing to difficulties in compounding a wash suitable for commercial use, for some eight or ten years after this work was completed. In the meantime, the high-boiling petroleum-oil washes were introduced and their uses and limitations investigated and the possibility of combining di-nitro-cresol with petroleum oil in a single wash has renewed interest in this substance. Quite recently, Shaw and Steer of East Malling, and Kearns and H. Martin of Long Ashton, have undertaken further studies of the toxicity of di-nitro-cresol in petroleum oil to the eggs of a considerable number of insects, and their results have confirmed the earlier work and added considerably to our information as to its high ovicidal power. The subject has also been taken up in the past two years by insecticide manufacturers, and spray-fluids containing di-nitro-cresol are now on the market as general winter washes intended for

control of capsid bugs and red spider as well as of aphides and apple sucker. It is too early yet to say how far preparations of this type will go towards solving the problem of the dual-purpose winter wash.

Another interesting development in connexion with possible winter washes effective against a wide range of insect eggs has followed studies of the ovicidal action of certain synthetic organic thiocyanates at Long Ashton, East Malling and Wye. Promising results have been obtained with preparations of dodecyl thiocyanate (lauryl rhodanate) and of β-butoxy-β'-thiocyanodiethyl ether (butyl carbitol thiocyanate) in petroleum oil as dual-purpose washes, and the evidence shows that these products are less phytocidal to developing buds and may safely be used somewhat later than di-nitro-cresol insecticides. One commercial spray-fluid of this type is now on the market and has been reported on by Hey. In this instance also, it is still too early to predict the place that these materials will finally take.

Raspberry beetle (*Byturus tomentosus* F.). Another fruit pest in regard to which the position has been completely changed is the raspberry beetle. It is the most injurious pest of raspberries and loganberries in this country and had become so serious that, about ten years ago, many growers were abandoning cultivation of these important crops, particularly where the fruit was sold in quantity for canning or bottling. The adult beetles may do much damage to the flowers and the larvae cause malformation of the fruit and loss of crop, their presence rendering the berries entirely unfit for canning. It had been found by Lees and Peren that a considerable measure of control could be obtained by three sprayings with lead arsenate during the blossoming period, but this was an expensive procedure and the use of an arsenical for this purpose was undesirable. Experiments with pyrethrum insecticides had also given promising results, but it was not until 1931 that the work of Steer at East Malling and a little later of Kearns and Walton at Long Ashton and of Petherbridge at Cambridge demonstrated that a thoroughly satisfactory solution of the problem was the use of derris insecticides, correctly timed and applied. Two applications of a derris spray were at first recommended, but more recent experiments showed that it is possible to control the pest satisfactorily by one thorough spraying towards the end of June to destroy the larvae, or by the use of derris dusts at about the same period. Good results are also obtained by the application of derris dusts at the beginning of the blossoming period in order to kill the adult beetles before egg-laying begins. The use of derris insecticides in one form or other for the control of this pest has now become routine practice among commercial growers; they are also widely used in gardens; and the raspberry beetle as a pest no longer presents any real difficulty.

A few words may be added here from a more general point of view about recent work on derris and allied plants as insecticides for they have come into widespread use in a quite remarkable manner in the last eight or ten years. They are specific in their action and some insects are resistant, but none the less they have found uses against many different pests of stock as well as of crops and they have the advantage of being non-poisonous. Derris insecticides have been known and have been on the market for a long time but did not seem to attract special attention until after the discovery and study of the active principle, or the most important active principle, present in the root—rotenone, and the demonstration of the astonishingly high insecticidal powers of this compound. Since then derris and other rotenone-containing plants, such as the South American *Lonchocarpus* species, have been the

subject of a great number of investigations, chemical and biological, to which English workers, particularly Tattersfield, J. T. Martin, and their colleagues at Rothamsted, and Cahn and his co-workers of the Cooper Technical Bureau, have made important contributions. The chemistry of these plants is exceedingly complicated, and different strains and varieties of the roots differ considerably in the proportions of the various compounds present; although samples with high rotenone content are necessarily powerful insecticides, the efficiency is not always correlated with the percentage of rotenone present, and the relationship between chemical composition and insecticidal efficiency has not yet been fully ascertained. Information is, however, gradually accumulating as a result of these researches which will, no doubt, lead to the formulation of a satisfactory procedure for the determination by chemical methods of the insecticidal value of samples. Methods of analysis applicable to all types of root are needed before standardization of these insecticides can be considered.

Apple and plum sawflies (*Hoplocampa testudinea* Htg. and *H. flava* L.). Reference must also be made to the successful development of means for dealing with two other important pests of fruit, the apple and the plum sawflies, which in certain districts and in certain seasons were causing serious losses and for which no satisfactory control measures were known.

The apple sawfly claimed special attention in the years 1929–34. It had become evident, from studies of the biology of the insect by Petherbridge and by Miles, that control by spraying was only likely to be successful if it could be timed to coincide with a very short period in the life history when the eggs were hatching and before the young larvae had burrowed into the developing fruit. As the result of further work by these investigators and by Kearns and others, it was shown that very thorough spraying with a nicotine wash applied at this period was highly effective. As a general rule, the right time for spraying is shortly after the blossom petals have fallen, but the best date varies somewhat in different years and districts, and a method of determining very closely the correct time in any particular locality has been worked out.

With regard to the plum sawfly, Petherbridge and Thomas, writing of this insect in the Eastern Counties in 1936, said that it had recently caused serious damage and "is now responsible for bigger losses than any other plum pest". The intensity of attacks varies greatly, but the pest is distributed throughout the country and is liable to become epidemic in any area in which plums or damsons are grown. The biology and control of this insect has been investigated by a number of workers between 1933 and 1937, and Petherbridge and Thomas, in a series of spraying trials in commercial plantations, showed first that good results could be obtained by means of a derris wash with spreader and later, following up some German work, that a spray containing quassia extract and soap was equally if not more satisfactory. Careful timing of the treatment is again of great importance and the washes should be applied when the covering of the young plum fruits is beginning to split. A derris-petroleum oil emulsion is recommended when red spider or plum thrips are troublesome as well as the sawfly, but for the latter only a quassia spray may be used.

The instances so far referred to have all concerned fruit pests. Turning now to pests of other crops, one or two examples may be given of interesting advances in practical control measures following recent work on several troublesome and destructive

Pests of vegetable crops

The cabbage root fly (*Delia brassicae* Bché.) is a widespread and common pest often causing severe injury to Brassicae of all kinds, both on a field scale and in allotments and gardens.

A good many years ago, co-operative experiments in which advisory entomologists and others took part demonstrated the usefulness of naphthalene and of corrosive sublimate (mercuric chloride) in checking losses due to this pest. Corrosive sublimate, though effective in many instances, is a most undesirable substance for general use, since it is so excessively poisonous and, in the past two years, Wright, at Cambridge, has been successful in obtaining very good control with calomel (mercurous chloride), a substance first employed against vegetable flies in America. The insecticide is applied in the form of a dust containing 4% of calomel, and it is found that two treatments, the first about the end of April and the second a fortnight later, entirely prevent any serious loss of plants. The dust is not difficult to apply, is harmless to the plants, and is relatively non-poisonous.

Wright has also employed this material for control of the *onion fly* (*Delia antiqua* Meig.), applying the dust along the rows of seedlings, and has obtained most satisfactory results. He has found too that effective control of onion fly is given by another method in which the seed, before sowing, is mixed with an equal weight of calomel itself, the chemical being stuck on the seeds by the addition of a little flour paste. The economic value of these treatments for both pests in normal times in unquestionable, and even under present conditions, when the price of calomel, in common with all mercury compounds, has greatly increased, there is little doubt that they will still prove well worth while.

New ways of dealing with *flea beetles* (*Phyllotreta* spp.) attacking Brassicae, particularly in seed beds, have also been found. Unusually severe attacks of these pests were experienced in some of the years between 1929 and 1934, and two or three resowings were necessary in many instances before a plant could be obtained. The experiments of Petherbridge and Thomas, and of Miles, with various insecticides in dust form, have shown that both derris dusts and naphthalene-silica dusts, applied just before the seedlings appear above ground, and at intervals later as may be necessary, provide an economic means of preventing losses. The older methods of control cannot be relied on to give consistent results, and the discovery of the effectiveness of these dusts is proving of great value to vegetable growers.

Slugs. Some advance in the last few years may also be noted in regard to means for dealing with the ubiquitous slug, though this has been more the result of chance observations than of any systematic experimental work. I refer to the now popular and generally recommended bait containing metaldehyde, usually in the form of powdered "Meta-fuel" mixed with bran, which has been found useful not only in gardens and allotments but also on a farm scale on cereals, potatoes and other crops. Metaldehyde, though dangerous to health if eaten, is not officially regarded as a poison and is certainly a much safer substance for general use than Paris green, the active constituent of the alternative and older poison bait: also it seems to have some advantage in efficiency, at any rate under some conditions, and certainly the results are more spectacular. The effect is to cause copious excretion of slime and the slugs are found in a moribund state from which, under normal out-door conditions, they do not often recover. Nothing is known of the real mode of action of metaldehyde and it is still uncertain whether it is a contact or a stomach poison. Curiously, it seems to be specific in its action and only Mollusca are affected; the bait is quite ineffective against soil insects such as wireworms and leather-jackets and also against woodlice. Information as to the effects of related aldehydic compounds would be of much interest and, at the moment, a really satisfactory substitute for the bran used in the bait is desirable.

It is a little difficult to know why Meta-fuel should have been tried as a slug poison.

I made an effort to find out who was really the discoverer with a feeling that his name should be on record and credit given where it was due. The trail, however, led rather far afield. So far as I know, the first mention of the bait to appear in print in this country was in a letter from Mr Hadden in one of the gardening papers in 1936. On enquiry from him, I learned that he had been told of it by a lady in Hampshire, and this lady kindly informed me that she had it from a friend in South Africa—another lady gardener. At this stage I came across a note referring to Meta as a control for slugs in the *Rhodesia Agricultural Journal* copied from a Press Notice issued by the Union of South Africa Entomological Department, and I got into touch with the writer of this note, Mr G. C. Haines, then stationed at Port Elizabeth. From him I learned that the Meta bait had been known at any rate to some people in South Africa since about 1934, and he was good enough to take considerable trouble to try to trace its origin. Several clues failed to lead to anything very definite, but finally the editor of a South African paper, *The Farmer's Weekly*, stated that a Mr C. A. Lowe had been the first to recommend the bait in the columns of that Journal. Unfortunately, on being asked if he could give the precise reference, the Editor replied that "a prolonged search had failed to bring the item to light, and although we have reason to believe that Mr Lowe was the first to recommend it to our readers, we are unable to state positively that he was its discoverer". There the matter rests at present, but two stories of the way in which the effect of Meta upon slugs was discovered are worth mentioning. The first is of a lady in South Africa who had been to a picnic in her car and had used Meta-fuel to boil a kettle: on returning home, the car was cleaned out and remains of Meta, as well as crumbs of food, were swept on to the ground; it was observed that they attracted slugs in large numbers. The second is similar, and relates to a lady who was using Meta-fuel to heat her curling tongs and threw the remains out of the window, afterwards observing an assemblage of dead slugs.

Hot-water treatment of certain plants

I want now to refer to a pest control method of rather a different kind.

Treatment of plants with warm water under carefully regulated conditions has provided a means of dealing with several pests, the control of which formerly presented great difficulties; and the development of this method has been to a great extent due to English entomologists. As long ago as 1913, Fryer, investigating possible means of killing the larvae of narcissus flies (*Merodon equestris* F. and *Eumerus* spp.) in daffodil bulbs without injury to the bulbs, found that this could be done by immersion of the dormant bulbs in water at 110° F. for 1 hr. At that time, the stem and bulb eelworm (*Anguillulina dipsaci* Kühn) had not been recognized as causing serious injury to narcissus, but a few years later Ramsbottom showed that this pest also could be controlled by the same treatment if the period of immersion was extended to 3 hr. Other pests, including the more recently discovered bulb scale mite, are also destroyed. The method has now become a routine procedure by bulb growers, and the eelworm is so serious a pest that it is doubtful whether commercial growing could be carried on if this or some equally satisfactory method were not available.

The application of hot-water treatment is, however, not without difficulties, and there is risk of unsatisfactory results unless certain details are attended to carefully. There is not a large margin, particularly with some varieties, between the temperature that the bulbs can stand without injury and that which will effectively destroy the pests. Careful regulation to

ensure that the correct temperature is reached in the bath and maintained for the proper time is therefore essential, and a recent study by Staniland and Barber at Seale Hayne Agricultural College of the factors to which poor results are likely to be due has led to valuable practical recommendations which should result in improving the performance and reliability of bulb baths. Another important point, if risk of injury is to be avoided, is to make sure that bulbs are as nearly as possible at their most dormant stage when treated and this period varies with different varieties and in different districts. This subject also has been under investigation at Seale Hayne College, and Wood at the Kirton Experimental Station has studied the effect of the temperature at which bulbs are stored before and after treatment in relation to injury.

In the last few years, the method has been applied for the control of certain pests of plants other than narcissus. In 1930, Hodson showed that the strawberry mite (*Tarsonemus pallidus* Banks) could be killed by warm-water treatment of the runners, and the conditions under which this can be safely carried out have since been further worked out by Hodson, Massee and Staniland. In 1935, Kearns and Umpleby obtained good results in freeing young apple trees (nursery stock) from woolly aphis (*Eriosoma lanigerum* Hausm.) by this method without damage to the trees. Hodson and also Edwards have applied it to the control of chrysanthemum eelworm (*Aphelenchoides ritzema-bosi* Schr.), and Staniland has shown that gladiolus corms can be treated and also violets and other plants under certain conditions. Further work will doubtless extend the range of usefulness of hot-water treatment, a method which at any rate in a number of instances is applicable to pests living within the tissues of plants.

Leaving now pests of living plants, we may consider for a few minutes work carried out on the control of

Pests of dried fruits

Nearly all foodstuffs are liable to infestation by insects during storage, and the problems met with in attempting to control such infestations are numerous and difficult. For a number of years, these problems have been the special concern, in this country, of the Department of Zoology at the Imperial College under Prof. Munro, and interesting practical results have followed from the work carried out there. A good instance is the investigations that have led to the development of satisfactory methods of control of moths of the genera *Ephestia* and *Plodia*, the larvae of which are extremely serious pests of dried fruits and other products.

The problem presented was how to deal not only with infested fruit itself which was arriving in London from Australia or elsewhere, but also with the barges in which it was transported from the ships to the wharves and the warehouses in which it was stored. Life-history studies of the moths *Ephestia elutella* Hb. and *Plodia interpunctella* Hb. had shown that both species normally pass the winter as full-fed larvae and pupate in any convenient shelter, and the warehouses provided endless suitable sites in walls and roofs and similar situations, the moths emerging in the following year, laying their eggs on fresh consignments of fruit, which, even if clean on arrival, rapidly became attacked.

Efforts had been made for some time by the Australian Dried Fruits Board to get the pests under control by fumigation of the fruit on arrival, but without great success, and the state of affairs had become so serious that it was even doubtful whether importation could be continued. Examination of the conditions revealed that the mixed fumigant in use was

unsatisfactory and inefficient, and experiments were put in hand to find something better, bearing in mind that the materials must not taint the fruit, nor leave any poisonous residue, nor present serious risk of fire. Page and Lubatti had been studying the behaviour of a number of fumigants under different conditions, among them ethylene oxide and, in collaboration with entomologists, undertook trial fumigations of loaded barges with this substance. Satisfactory distribution of the fumigant was one of numerous difficulties met with, but this was overcome by devising a special form of vaporizer and finally successful results were obtained. The method has been adopted by the commercial interests concerned, and the fruit is now free from infestation on arrival at the warehouses.

This did not, however, complete the matter; it was necessary also to destroy the insects in the storage buildings in order to avoid reinfestation of the fruit. Here, too, fumigation was the first method considered, but it was evident that very great practical difficulties would be met in attempting to fumigate large warehouses, some of them old buildings almost impossible to render even approximately airtight. Moreover, although fumigation is a very old method of insect control and widely employed for many purposes, accurate information on many of the most important factors affecting the efficiency and safety of the process under different conditions was lacking. Page and Lubatti had in hand and have since continued a systematic study of the application of fumigants, working particularly with hydrocyanic acid and ethylene oxide. A more satisfactory method than was then available for taking samples of the mixture of air and fumigant for determination of the concentration at different points was devised, and by means of this the actual dosage reached at any point in the space fumigated and the falling of the concentration with time under different conditions could be ascertained.

Problems concerning the best means of ensuring even distribution of the fumigant, of the rate of dispersal and penetration, of absorption by the goods under treatment and by the fabric of buildings, all points greatly affecting the efficiency of the fumigant, have also been investigated, and it is not necessary to dilate on the practical value of accurate data on these subjects. Application of the methods adopted and of the experience and information gained in this work, so far as it had then gone, to the question of the treatment of warehouses infested with *Ephestia* and *Plodia* led to the conclusion that the high cost and other difficulties made fumigation impracticable under these conditions, and a solution of the problem had to be sought in other directions.

Both adult moths and larvae were abundant in the warehouses and a liquid spray might be useful, though it could not be expected that every crevice harbouring larvae would be reached. The use of pyrethrum was suggested and the possibility of adopting this insecticide for the purpose was investigated by Potter. He found that an emulsion of pyrethrum extract and petroleum oil was moderately effective against the larvae but did not by any means do all that was required. He then decided to attempt in the first place to deal with the adult moths and tried spraying a concentrated preparation of pyrethrins diluted with a highly refined white oil in atomized form. Experiments with this were remarkably successful and apparatus for producing the atomized spray suitable for use on a large scale was then constructed. By this means a mist-like spray was produced, the minute particles remaining suspended in the air for some time and penetrating well. Not only could the adult moths be destroyed by this means but it was also found that larvae crawling over the surfaces exposed to the spray were killed. By spraying frequently, it was possible to free badly infested warehouses completely

from the pests and to protect them and goods stored in them from reinfection. The insecticide is non-poisonous, non-inflammable and does not taint or cause injury to foodstuffs, and its use is economically practicable and has in fact become a routine practice.

The protection of goods from infestation by this type of atomized spray is of particular interest, and Potter has also investigated this effect and its practical applications. He found that the spray formed an excessively fine invisible film over the surfaces on which it settled, and that this film was toxic and remained toxic for some time to various insects exposed to it. Using the pyrethrum-white oil mixture, containing 0·8 % of pyrethrins, a single good spraying resulted in the deposit of a film which paralysed and eventually killed *Ephestia* and *Plodia* moths exposed to it for not more than 30 min., and larvae exposed for 1–24 hr. depending on the age of the film. Other indoor insects were similarly affected in varying degree. Experiments also showed that the oil alone used in this way had very little toxicity. Larger scale trials demonstrated that this film-spraying method can be applied under commercial conditions as a preventive of infestation of dried fruits and other products in storage. Periodical spraying during the period of emergence of the moths is found in practice to give continuous protection; it can be carried out without disturbance of the goods and is cheaper than fumigation and is not dangerous to health. As Potter has pointed out, the method may prove to be applicable to the control of certain other insects and further work may show that other insecticidal substances can be utilized similarly.

Pyrethrum

Reference has just been made to a comparatively new use for pyrethrum, and I propose, finally, to say something about recent general lines of work on this insecticide. Perhaps its most important use is in the form of fly sprays, but pyrethrum insecticides are non-poisonous and have great advantages for certain horticultural purposes; they are largely employed in gardens, in glasshouses and for special purposes such as the control of mushroom pests; and in addition, as already mentioned, they are finding application in connexion with some pests of stored products. Pyrethrum flowers are one of the oldest known insecticides, but it is only in the last fifteen years, since the discovery by two Swiss chemists of their active principles, the pyrethrins, that accurate and detailed information about them has become available. The subject has attracted the attention of research workers in England as elsewhere and both laboratory and field investigations have been undertaken.

Tattersfield, J. T. Martin, and their colleagues at Rothamsted have studied the toxicities of the active principles, pyrethrins I and II, and have worked out methods of analysis for their determination in the flowers which have been widely adopted. Correlation of the figures obtained with the results of biological tests on insects has proved the usefulness of these methods as chemical means for the evaluation of samples. The quantitative development of the pyrethrins during growth of the flower heads has also been followed, and it was found that the content of pyrethrins increases from the small bud stage up to the time when the flowers are fully blown. After that stage there is a fall in the percentage of pyrethrins due to the increase in the weight of the seed following fertilization. These findings were of much practical importance, for they indicate that the proper time to take the harvest is when the flowers are just fully open. Formerly, it had been an established convention of the trade that pyrethrum flowers should be picked well before they are fully blown. Among other sides of the subject investigated by the Rothamsted workers have been the

effect of environmental conditions on the growth of pyrethrum, the stability of extracts and emulsions, and the cause of the loss of activity of pyrethrum dusts exposed to sunlight and air and the possibility of stabilization. Experimental work on artificial drying of the flowers, in collaboration with Jary at Wye, showed that temperatures between 50 and 60° C. for 10 and 6 hr. respectively, are suitable.

Pyrethrum has been the subject of a good deal of work from quite another aspect—the possibility of its cultivation as a crop in this country. Experiments were begun in 1925 by the Plant Pathological Laboratory of the Ministry of Agriculture when small plots were laid down at a number of places throughout the country on various types of soil. These trials, carried on for a number of years, demonstrated that the crop can be grown and harvested successfully under our conditions and that a satisfactory yield of dried flowers can be obtained. In co-operation with the Rothamsted investigators, samples of the flowers from many of these plots were tested by both biological and chemical methods and their insecticidal efficiency was found to be at least equal to and in many instances higher than that of imported pyrethrum. Arrangements were then made to plant a series of larger areas of ½–1 acre in order to obtain more information about the costs of growing, harvesting and drying the flowers. These larger scale experiments confirmed that high yields of produce could be obtained under good conditions and that, even on poor soils, the crop did reasonably well. The costings data indicated, however, that at the rather low price of pyrethrum flowers then prevailing, it was doubtful whether the crop could be grown to pay; labour costs, particularly for cutting the flowers which, in the absence of a mechanical means of harvesting, had to be done by hand, appeared to be the most important item of expense. At the present price of pyrethrum, about treble what it was when these experiments were done, this conclusion could safely be revised, and the work has demonstrated that, if it becomes desirable to do so, supplies of pyrethrum could be home-produced.

In other experiments, in co-operation with the investigators at Rothamsted, it was shown that individual plants vary greatly in pyrethrin content and in cropping capacity, and there is thus a prospect of producing flowers of improved quality. A strain grown at the Ministry's Plant Pathological Laboratory proved to be of higher quality than imported material which at that time came in commercial quantities only from Japan and Dalmatia. In 1929, some of this seed was supplied to the Department of Agriculture in Kenya and has played a considerable part in the development of the now flourishing pyrethrum-growing industry in that country. Though pyrethrum is a plant of temperate regions and fails to produce flowers under really tropical conditions, it can be grown successfully on the Kenya Highlands at altitudes of 5500 ft. and upwards. Under these conditions, the plants flower practically continuously for 10 months of the year and pickings of open blossoms are taken every 10 or 14 days. Kenya growers were looking for a new crop and large areas were planted; the production of dried flowers, which in 1934 was 41 tons, has rapidly increased and in 1938 reached nearly 1800 tons. Steps have been taken to prevent the importation of inferior strains of seed, and under an Ordinance passed in 1935, the sale of pyrethrum is regulated and is in the hands of a single agency, the Kenya Farmers' Association. Kenya pyrethrum is now sold with a guaranteed minimum pyrethrin content and commands a substantial premium on the market.

In the past ten years small supplies of seed have also been sent on request from the Ministry's Laboratory to the Agricultural Departments of most of the Dominions and

Colonies and to India, for cultivation trials. Successful results have been obtained in some places and the crop is now grown commercially to a small extent in several parts of the Empire, though nowhere else on the scale reached in Kenya.

Pyrethrum is an insecticide for which there is an increasing demand and it is satisfactory that the position has been improved, both with regard to the quantity and quality of supplies.

I will not add further to the instances chosen for brief review of investigations from which results directly affecting insect control in practice have followed, though it would not be difficult to do so. No mention has been made of the work of W. R. Thompson and his staff at Farnham House Laboratory on insect control by parasites and predators, of developments in the control of timber insects and the preservation of timber from attack at the Forest Products Research Laboratory, of work on combined insecticides and fungicides at Long Ashton and East Malling, of work on the improvement of spraying machinery at Wye—all important contributions to the solution of practical problems—and the list could be extended. The number of workers in this branch of applied biology is small compared with that in some other countries, but progress has been made and some results obtained not without value to the community. None are more conscious, however, than the entomologists and chemists concerned that only a beginning has been made and that there is an abundance and variety of practical problems, some of them of long-standing importance, demanding attention.

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THE GROWTH AND ANATOMICAL STRUCTURE OF THE CARROT (*DAUCUS CAROTA*) AS AFFECTED BY BORON DEFICIENCY

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(With Plates 7 and 8 and 7 Text-figures)

THE statement that boron is essential for growth of the carrot (Warington, 1937) was corroborated by Tiedjens & Schermerhorn (1938), and Schropp (private communication).

Tap-rooted crops appear to be particularly sensitive to boron deficiency, and much information on the subject has been published regarding sugar beet and mangolds (Chenopodiaceae), and turnips and swedes (Cruciferae). Little is known of the response of umbelliferous plants, and although Purvis & Ruprecht (1937) showed that boron was needed for the healthy growth of celery, Rigg's tentative results with parsnips (1937) had not come to the author's notice when the present experiments were started.

EXPERIMENTAL PROCEDURE

Carrots were grown in solution culture until the flowering stage was reached, and material was preserved at successive phases of growth for anatomical investigation. Preliminary trials with three media were carried out, and as a Van der Crone solution[1] gave the best results it was used throughout. The seed (Sutton's Scarlet Horn) was sown in sawdust on 4 March 1937, and the seedlings, six together, transferred on 24 March to small culture flasks filled with the appropriate nutrient solution, as experience had shown that very small seedlings do not thrive in large culture vessels. After a further 2 weeks they were set up singly in culture bottles of 600 c.c. capacity, seven different concentrations of boric acid being tried, ranging from 1/50,000 to 1/5,000,000. Five replicates were selected for each treatment, including five controls without boron. Renewal of the solutions was carried out approximately every 2 weeks.

In the control plants signs of deficiency appeared by 8 June. Growth was stunted, and the leaves dried, turned a reddish or yellow colour and pointed downwards in a characteristic manner (Pl. 7, fig. 3). This habit may prove a useful diagnostic character in the field, as it appears during the early stages of deficiency, while the plant is still capable of recovery if boron is supplied. There is no danger of confusing this with the natural downward drooping of the older leaves, as erect new growth is then also present (Pl. 7, fig. 1). The laminae of the youngest leaves were often reduced to little more than the midrib in the absence of boron (Pl. 8, fig. 5), and shedding of the outer leaves was common. Eventually the central growing point died and there was an increased development of lateral buds. Tap-root formation started normally, but the laterals became short and thick and did not form the usual fine tassel. Progress in the symptoms was slow, but by August or September death was generally complete.

[1] KNO_3 1·0 g.; $MgSO_4$ 0·5 g.; $CaSO_4$ 0·5 g.; $Ca_3(PO_4)_2$ 0·25 g.; $Fe_3(PO_4)_2$ 0·25 g.; $MnSO_4$ 0·001 g.; H_2O to make up 1 l.

Provision of boric acid within the range 1/2,000,000 to 1/50,000 of culture solution resulted in perfectly healthy growth, and the plants receiving the different quantities were indistinguishable. Development was somewhat retarded when only 1/5,000,000 was supplied, though with the regular renewal of the solution symptoms of deficiency did not appear. No evidence of toxicity was obtained when the larger quantities of boron were given.

Owing to the early death of plants completely deprived of boron, and the fact that the carrot normally does not flower until the second season, it was not possible to determine the effect of a deficiency of this element at the flowering stage, except by withholding it from plants already given a supply during the earlier stages of development. Two plants in each boron treatment were accordingly transferred to a nutrient solution without boron at the end of October, the three remaining plants continuing to receive their original amount. All solutions were renewed regularly as before. It was thus possible to compare the efficacy of different initial supplies of boron in preventing the onset of deficiency symptoms, and, further, to study the gradual changes that occurred between plants that had previously received identical treatment. The latter is illustrated in Pl. 7, figs. 1, 2. Fig. 1 shows two plants photographed in October 1937, each of which had hitherto received 1 p.p.m. boric acid. After this date boron was withdrawn from one. In the following spring, fresh growth occurred only on the plant still receiving boron, and by June this was in flower, whereas the other was dead (Pl. 7, fig. 2). In general, when the initial supply was 1 p.p.m. boric acid or less, deficiency symptoms had developed by mid-March, but they were delayed till mid-April or May when the original amount given was greater. Within the limits of this experiment the larger the initial supply of boron the longer the onset of deficiency symptoms was delayed, though all plants eventually showed them in some degree (Pl. 7, fig. 4).

Where the preliminary dose of boron was sufficiently high, erect flowering shoots were formed, but when the supply of boron was used up, these ultimately died at the apices, and the heads bent over. The symptoms in this case differed in one important respect from those appearing earlier in the life history of the plant. Whereas the main shoot usually shows the effects of the deficiency first, death of the smaller side shoots following later, this sequence was reversed when the lack of boron did not arise until the flowering heads were developed (Pl. 8, fig. 6).

The tap roots were unexpectedly firm when the plants were harvested on 10 June: softness was only characteristic of plants deprived of boron, i.e. those which had grown less in the second season and consequently made smaller demands on the food reserves of the root. A faint sickly smell was often associated with these soft roots suggestive of breakdown of storage products, but it was not possible to carry out any chemical investigations.

Small clusters of wart-like excrescences were noticeable on the tap roots of many older plants deprived of boron, the nature of which will be discussed later. All the carrots were cut open, but there were no obvious signs of internal breakdown. A few of the no boron plants showed slight blackening at the bases of the leaves, and in one instance the vascular core was outlined by a black streak. Microscopical investigations disclosed certain abnormalities which are described below.

Anatomical investigations

Intensive study has been made by a number of authors of the effect of boron deficiency on the structure of sugar beet and turnip. The symptoms, at least in the first year of growth, are not identical, the differences being closely associated with their respective anatomical conformations. The carrot "root" resembles the turnip in the general arrangement of its tissues, but differs from it in the relative proportion occupied by the various components, while both differ fundamentally from the sugar beet. Turnip and carrot "roots" consist of a combination of swollen hypocotyl and root proper, the internal structure being that of a typical dicotyledon with a single cambium zone. The carrot has a wide cortex, the cambial layer lying well below the surface of the "root", whereas in the turnip the cortex is narrow, the cambium being close under the epidermis. The central zone is occupied by woody tissue in both cases, that of the turnip being the less lignified and composed mostly of wood parenchyma. Both these "roots" differ fundamentally in their structure from the sugar beet or mangold, where a series of cambia arise from the pericycle in a centrifugal direction, and where vascular tissue is dispersed in layers throughout the root alternating with parenchyma. These structural differences are important in following the effects of a boron deficiency, e.g. Rowe (1936) has in this way accounted for the fact that although the central heart leaves of the sugar beet die first, breakdown of the tissue starts on the outer margins of the "root", since it is here that the newest cambia, associated with the youngest leaves, are situated.

In the swede and turnip, as Jamalainen (1935) showed, it is chiefly the wood parenchyma that is affected by a deficiency of boron, so that the breakdown of tissue is confined to the middle of the bulb. It is not clear why no accompanying symptoms appear at the growing point of the shoot, as is usual in most other plants, but the damage within the root may be extensive without any external evidence of the trouble. The reverse was the case with the carrot in the present experiments, for death of the shoot could occur unaccompanied by any visible breakdown of tissue within the tap root. These latter plants were grown in solution culture, and as Dennis & O'Brien (1937) pointed out, symptoms familiar enough to the farmer may not be obtainable under such conditions, as death ensues before the requisite growth stage is reached. Similarly, other symptoms may appear in solution cultures which are not seen in the field, i.e. there may be an appreciable difference between the effects of a shortage of an element and its complete absence.

A. *Shoot and tap root*

In order to get a progressive picture of the effect of boron deficiency on carrots, plants were set up in nutrient solution in September 1937, and carried on until August 1938, fifty-six of the total 178 plants receiving boron. Van der Crone's solution was used, and where boron was supplied it was added as boric acid at the rate of 1 p.p.m. nutrient solution. Early growth was good considering the time of year, but rather slow during the winter months, as although sufficient heat was supplied to the glasshouse to guard against frost, no attempt was made to force the plants or to provide artificial illumination. Nutrient solutions were renewed every 4–5 weeks up to the end of May, and every 2–3 weeks from then until August. This appeared to be sufficient to maintain healthy growth. No flowering occurred, but well-formed tap roots developed on all plants whether or not they eventually died from a lack of boron. Material was preserved[1] at intervals from plants selected at random, so that

[1] Alcohol (70%) 100 c.c.; corrosive sublimate 3 g.; acetic acid (glacial) 3 c.c.

comparison was possible between those grown with and without boron at progressive stages of development. The first sample was taken when the plants were 2 months old, just as the first swelling of the tap root was noticeable. At this stage, and at the time of the second sampling 3 weeks later, no differences were apparent between the plants grown with or without boron in size, external appearance or internal structure. When the plants were 11 weeks old and diameter of the roots approximately 0·75 or 1·0 cm., signs of internal breakdown began to appear where boron was not supplied, though as regards size and external appearance they still closely resembled the plants receiving boron. The deficiency symptoms at this stage consisted chiefly of hypertrophy and collapse of the walls in the parenchyma associated with the vascular tissue close to the growing apex of the shoot (Text-fig. 1). The tip was unaffected as were also the older vascular bundles, but some of the cortical cells and young leaves showed patches of disorganized tissue (Text-fig. 5). By 2 February the shoots of several of the plants without boron were stunted and their leaves curled back, though their roots were still normal in size. All showed some internal breakdown even though they appeared healthy externally. In certain cases the meristem at the tips was abnormal (Text-fig. 4), in others the trouble was confined to a small zone behind the apex, being noticeable in a few outgoing leaf traces or in the cortex, but all types of injury could occur together. This localization of the area involved necessitated cutting a long series of sections before it was safe to conclude that the internal structure was normal. In some cases breakdown of tissue was found at the base of young unfolded leaves surrounding the apical bud (Text-fig. 7). Premature leaf shedding is a recognized symptom of plants suffering from a lack of boron, but lesions in such immature leaves were unexpected.

All components of the vascular tissue were liable to be affected in the absence of boron. Though the most obvious symptom was patches of collapsed and blackened parenchyma, enlarged and irregular cambium and phloem elements generally occurred, and many of the xylem vessels were blocked (Text-fig. 3). Hypertrophy was also common in the mesophyll of the leaf and ground tissue of the cortex. The resin canals closely associated with the vascular bundles in the leaves and flowering stems were also affected, the walls of the cells collapsing and the regular outline of the canal being lost (Text-fig. 2). Breakdown was rare in the centre of the plant, whether this consisted of a parenchymatous pith as in the shoot, or of lignified xylem elements as in the root proper. In this respect the carrot afforded a contrast to the turnip where the middle of the bulb showed the most severe symptoms.

By 4 March all the plants without boron showed signs of dying at the apex of the shoot, and after a further 2 weeks their roots were noticeably smaller than where boron was supplied. The progress in internal disorganization was slow. As it seemed probable that death of these plants grown without boron would soon take place, a modification of the original scheme of the experiment was made. Plants were transferred, on three successive occasions, from the control solution to one containing boron, similar transfers from a with-boron to a no-boron solution being made at the same time. Others were temporarily supplied with 1 p.p.m. boric acid for a month and then replaced in the solution without boron. In this way it was possible to determine both the course of recovery from the deficiency, if and when it took place, and also the effect of the deficiency at a later stage of growth than would otherwise have been available.

A temporary supply of boron for 1 month in the early spring (February–March) improved growth very markedly, and new roots and shoots developed. On the removal of the boron

supply fresh deficiency symptoms set in rapidly, and by the end of May were more pronounced than in plants of the same age which had never received boron. The temporary stimulus to growth had presumably increased the nutritional demands and hastened the utilization of the available supply of boron.

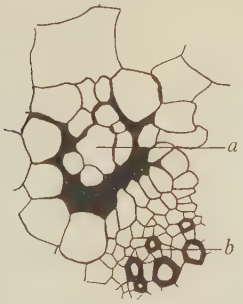
When the addition of boron was continued, the effect depended both on the length of time the plant had previously been deprived of it, and the period allowed for recovery before sampling. A supply of boron from March to May resulted in vigorous new growth of shoot and root, a sharp distinction being noticeable between the lateral roots developed before and after the boron was supplied. Whereas the former were short, thick and discoloured, the latter were long, fine and white. Sections from these plants showed that the younger parts of the shoot, viz. the inner leaves and central growing point, were healthy, though the older leaf traces, lateral buds and the cortex of the tap root below the shoulder bore definite signs of the earlier period of deficiency. What appeared to be the chief growing point was probably a lateral bud which had assumed the central position on the death of the original apex of the main stem, the latter being represented by an unhealthy bud in a lateral position. Extension of the supply of boron until August resulted in further recovery, though slight traces of the previous lack of the element could be found. If boron was withheld until May vigorous recovery could occur, and in one instance fresh growth started after an application of boron in June. Both these sets of plants were carried on until August, and on being sectioned showed but slight signs of their long period without boron.

When boron was withdrawn after it had been supplied during the winter and early spring, a considerable time elapsed before deficiency symptoms manifested themselves, though there was much individual variation in this respect. If the transfer to the boron-free solution was made in March, leaf curling appeared in some cases by the end of May, together with breakdown in the central vascular tissue and in the young leaves. The meristem of the growing point, however, remained healthy. Other plants treated similarly but kept on until August showed little advance in internal deficiency symptoms of the shoot, though the roots became thickened and were badly disorganized in structure. Removal of the boron as late as May or June did not allow time for marked signs of deficiency to develop before August, though there was a lack of the usual new growth in the shoot, and in some cases the rootlets began to thicken at the tips. Internally, some collapse and blocking of the vessels in the youngest leaves occurred.

B. *Lateral roots*

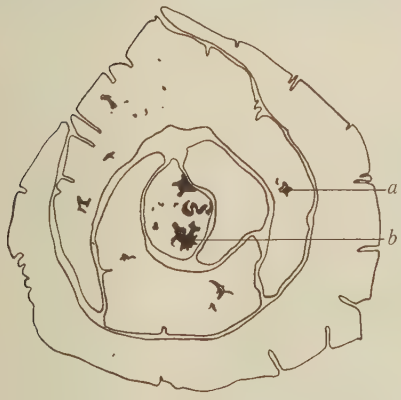
The lateral roots of carrots grown without boron were short and thick, swellings occurring in places, particularly at the tips. Sections showed that the swollen areas were due to the apparent fusion of two or more rootlets, and that the undifferentiated tissue at the growing apex was frequently in an unhealthy condition. Branching had evidently begun, but the new rootlets had failed to emerge, as was found in *Vicia faba* plants deprived of boron (Warington, 1926). Although the apical growth in both parent and lateral rootlets was checked, extension in a radial direction continued, and it was common to find two roots with considerable secondary thickening within a single cortex (Pl. 8, fig. 7). The formation of the wart-like excrescences on the older tap roots could be accounted for in a similar manner, as they arose from a grouping, in a telescoped region, of much-branched laterals whose apical growth and proper emergence had been checked (Text-fig. 6).

Text-fig. 1.



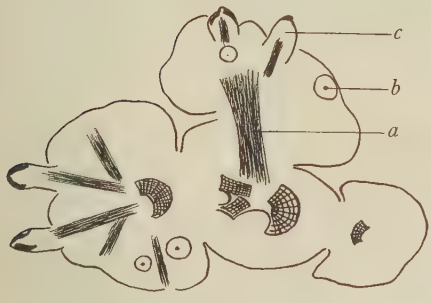
Text-fig. 2.

Text-fig. 3.

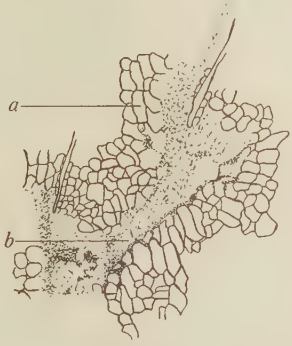


Text-fig. 4.

Text-fig. 5.



Text-fig. 6.



Text-fig. 7.

Text-fig. 1. Longitudinal section of vascular bundle behind shoot apex showing (*a*) hypertrophy and (*b*) breakdown of thin-walled tissue.

Text-fig. 2. Transverse section of resin canal showing (*a*) breakdown of canal walls, (*b*) associated vascular bundle.

Text-fig. 3. Transverse section of vascular bundle in leaf showing (*a*) blocking of xylem vessel, (*b*) blocking in phloem, (*c*) hypertrophy, (*d*) breakdown of xylem parenchyma.

Text-fig. 4. Transverse section of young shoot showing patches of broken down tissue in (*a*) leaves and (*b*) growing apex.

Text-fig. 5. Longitudinal section of young shoot showing (*a*) patches of broken down tissue in leaves though actual growing point (*b*) is healthy.

Text-fig. 6. Diagrammatic sketch of section through wart-like growth on tap root showing abnormal branching. (*a*) vascular strand from parent root, (*b*) longitudinal and (*c*) transverse sections of lateral roots with apices breaking down.

Text-fig. 7. Longitudinal section of (*a*) young leaf showing collapsed tissue (*b*) at base.

C. *Flowering stem*

The normal flowering stem of the carrot consists of a ring of vascular bundles within a narrow cortex and surrounding a large thin-walled pith. Characteristic features are the groups of collenchyma forming ridges on the outside of the stem, and the numerous resin canals in the cortex. A deficiency of boron first showed itself in a breakdown of the tissues nearest to and including the vascular ring, all components of which, but particularly the cambium, were liable to be affected. Patches of degeneration also occurred in the cortex, and later the pith became involved. The severity of disorganization in the pith was noteworthy, as in the vegetative stage this part of the stem and hypocotyl was little affected. An advanced condition of boron deficiency is shown in Pl. 8, fig. 8: disintegration of the vascular tissues is almost complete only the thick-walled xylem elements retaining their identity.

Summary

Carrots grown in nutrient solution without the addition of boron were unable to complete their normal development and exhibited both external and internal deficiency symptoms: 1 part boric acid in 2 million was sufficient to maintain healthy growth when the culture solutions were renewed regularly.

The effect of a lack of boron at late stages of growth was determined by transferring plants to a boron-free solution after they had received an initial supply.

The chief deficiency symptoms were:

(*a*) *External.* The leaves curled back, pointed downwards and often turned red or yellow. The laminae of the youngest leaves were much reduced and the growing apex of the shoot died. The flowering stage was not reached. A small tap root was formed but the laterals were abnormal in appearance, being short and thickened in places. When the boron deficiency only arose in the later stages of development, certain additional symptoms appeared, viz. the flowering heads bent over and died, those of the side shoots succumbing first. In some cases small wart-like excrescences arose on the outside of the tap root.

(*b*) *Internal.* Although no marked degeneration within the tap root was evident to the naked eye, microscopic investigation showed that most of the tissues of the shoot and root were affected by a lack of boron. Breakdown began in the region of the growing point of the shoot, patches of irregular and blackened tissue appearing in the young leaves and in the vascular tissue close behind the tip, though the meristematic apex itself was not necessarily first involved. Later, similar patches were found in the cortex and occasionally also in the pith. Hypertrophy usually preceded or accompanied the formation of these lesions. All components of the vascular bundles were affected, blocking occurring in the xylem and phloem, and hypertrophy and breakdown in the thin-walled tissues. The formation of the wart-like growth on the tap roots and the swellings on the laterals was explained by the fact that in the absence of boron, repeated branching and radial extension of lateral roots continued, when elongation and emergence were prevented through death of the apices.

Recovery took place in both shoot and root when boron was supplied, even after prolonged growth in its absence. Traces of the earlier deficiency period could be detected in the anatomical structure of the older parts of the plants although the external appearance was quite normal.

I am indebted to Dr H. G. Thornton for taking the photomicrographs and to Mr V. Stansfield for the rest of the photographs.

Fig. 1.

a *b*

Fig. 2.

a *b*

Fig. 3.

a *b* *c* *d* *e*

Fig. 4.

WARINGTON.—The Growth and Anatomical Structure of the Carrot (*Daucus carota*) as Affected by Boron Deficiency (pp. 176–183)

a *b*

Fig. 5.

a *b*

Fig. 6.

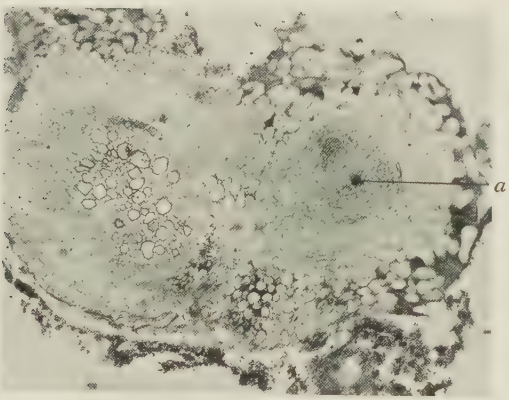


Fig. 7.

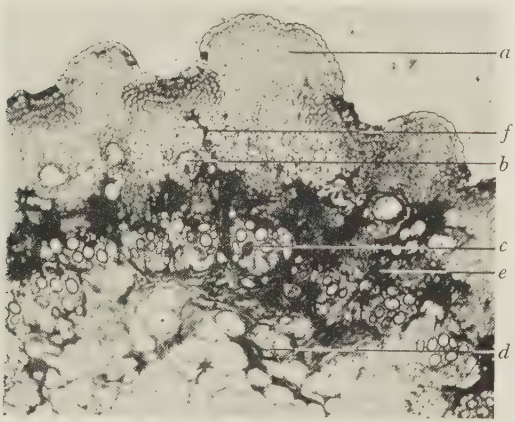


Fig. 8.

WARINGTON.—The Growth and Anatomical Structure of the Carrot (*Daucus carota*) as Affected by Boron Deficiency (pp. 176–183)

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EXPLANATION OF PLATES 7 AND 8

PLATE 7

Fig. 1. Carrot plants grown in nutrient solution containing 1 p.p.m. boric acid; photographed October 1937.

Fig. 2. The same plants as in fig. 1 photographed June 1938. (*a*) No boron since October 1937. (*b*) 1 p.p.m. boric acid throughout.

Fig. 3. Carrots grown in nutrient solution for 6 months. (*a*) Without boron showing characteristic position of the leaves. (*b*) 1 p.p.m. boric acid.

Fig. 4. Carrots photographed June 1938, deprived of boron since October 1937, previous treatment (*a*) 1 : 5 million, (*b*) 1 : 2 million, (*c*) 1 : 1 million, (*d*) 1 : $\frac{1}{2}$ million, (*e*) 1 : $\frac{1}{4}$ million boric acid. Appearance of deficiency symptoms progressively delayed as the initial supply of boron was greater.

PLATE 8

Fig. 5. (*a*) Normal young carrot leaf. (*b*) Lamina reduced when boron deficient.

Fig. 6. (*a*) Boron deficiency symptoms in carrot at the flowering stage. Side shoots bending over while the main shoot still healthy. (*b*) Plant of same age supplied with boron.

Fig. 7. Transverse section though swollen portion of lateral root of carrot deprived of boron, showing apparent fusion through incomplete emergence of new rootlet (*a*).

Fig. 8. Transverse section of flowering stem of carrot suffering from boron deficiency showing (*a*) collenchymatous tissue, (*b*) resin canal, (*c*) blocked vessel, and breakdown in (*d*) pith, (*e*) vascular ring, (*f*) cortex.

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SOME ROOT-FORMING SUBSTANCES, IN RELATION TO ONE ANOTHER, TO PLANT METABOLISM, AND TO GROWTH

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(With 1 Text-figure)

WITH auxin *a* and *b*, isolated by Went (1927)[1] and his colleagues, and explored chemically by Kogl *et al.* (1934), there occurred in urine a third substance, first named heteroauxin, but immediately recognized as 3-indolylacetic acid. The high activity shown by this compound when tested on the coleoptiles of oats, on the split stems of peas, and other plant tissues, led to tests of numerous synthetical substances, some closely related by their chemical structure to indolylacetic acid, others less so; the list of active substituted fatty acids was enlarged by Zimmerman (1935) and his co-workers to include α-naphthylacetic acid, now a substance of economic importance.

The ensuing practical application in horticulture of the root-forming activity of such compounds was appreciated and established several years ago (Tincker, 1936). Several synthetic growth substances, 3-indolylacetic acid, γ-3-indolylbutyric acid and α-naphthylacetic acid, are now available as pure substances or as proprietary articles, and may also be purchased in solution or dispersed in talc. They have been used with considerable success to facilitate the vegetative propagation of numerous cultivated plants by causing a greater number of stem cuttings to root more profusely in a shorter time than without treatment. Pearse (1939) lists approximately a thousand examples of cultivated plants which have responded favourably to stated treatments, and his index makes no claim to be complete. At Wisley the propagation of several hundred plants has been aided by growth substances used as a paste or in solution, or in powders (Tincker, 1938; Tincker & Unwin, 1939); and, in addition, the relationship of root-forming substances to compounds naturally occurring in plant tissues and to one another has been studied, as also the reactions of entire plants to these substances.

I. AMINO-ACIDS AND PRECURSORS OF GROWTH SUBSTANCES

A close relationship exists between the indolyl derivatives of the fatty acids and tryptophane, the significance of which in plant tissues is yet partially obscure. By deamination indolylpropionic acid is obtained; α-3-indolylpropionic acid has a high activity when applied to oat coleoptiles; tryptophane although inactive has a delayed action. Through the co-operation of Dr F. L. Pyman, tests, as root-forming substances, have now been made of the activity of acids derived by similar deamination from further plant amino-acids.

Method of testing

The method of testing prepared substances closely followed the technique used in plant propagation. Uniform cuttings were placed with their basal ends in dilute solutions for a given time, and after uptake of the substance the cuttings were washed and placed in coarse sand in frames. Ranges of concentrations were tested, at which indolylbutryic acid and

[1] See note regarding literature cited.

α-naphthylacetic acid proved efficient. These substances acted as standards, and by proving the responsive condition of the selected plant material permitted direct comparisons. Activity was judged by the induced formation of roots and by the development of a zone of stimulation in which an internal callus of undifferentiated parenchyma developed—thus the substances were assessed as root-forming substances and as inceptors of cell division in the cambial regions.

Derivatives of tryptophane (α-amino-β(3-indolyl)propionic acid) and other indolyl compounds
Standard substances.

Indolylpropionic acid has not been used in our tests but indolylacetic acid has been one standard substance. In tests made with cuttings of a wide range of plants at various seasons more rapid root formation has been obtained. This succeeds the stimulation of the cambial region and division of adjacent phloem parenchyma cells, such stimulation took place in winter during a period of normal quiescence. The compound was used at concentrations between 100 and 20 p.p.m.[1] in solution and dispersed in talc at 200 p.p.m.

Another indolyl compound, γ-3-indolylbutyric acid, has proved of practical value for plant propagation and has now given satisfactory results in our tests with more than 300 plants. Its effects closely resemble those of indolylacetic acid but it is more potent and evoked responses in certain cases where the former acid failed. The concentrations used ranged from 2 to 100 p.p.m. This acid is also effective in powder form. Indolylbutyric acid affords a high standard for comparative work with other active compounds.

Derivatives of tyrosine (β(4-hydroxyphenyl)alanine).

β-*p*-Hydroxyphenylpropionic acid was tested with four species including *Buddleja alternifolia* and *Lonicera yunnanensis* taken at suitable seasons. No root formation was induced, nor was cell division accelerated. The plant material responded to the other treatments with active substances used at a concentration of 50 p.p.m.

A similar series of tests was made with *p*-hydroxycinnamic acid using *Erica arborea*, *Lonicera nitida* and other plants, but no activity was observed. Positive responses were obtained from the series treated with active substances at 33 p.p.m.

Derivatives of phenylalanine.

β-Phenylpropionic acid (hydrocinnamic) was tested with four species including *Buddleja alternifolia*, *Lonicera yunnanensis* and *Tricuspidaria dependens* used at the appropriate season in each case. The activity observed was of a very low order, the results were barely significant. The substance was of little value for routine propagation purposes. Very little, if any, stimulation occurred as judged by induced cell division.

Phenylacetic acid has been tested in various ways at Wisley. Application of the substance in paste form to tomato plants produced epinasty and feeble root development. *Solanum capsicastrum* also responded to the application of a paste of 1 % in lanolin. Cuttings of *Schima argentea* and *Kalmia latifolia* formed more callus as the result of applications of phenylacetic acid. The threshold value for stimulation was much higher than that of indolylacetic or naphthylacetic acid, the latter substances will readily cause reactions in a tomato if applied in paste of 0·1 %, they are almost ten times as active as phenylacetic acid. Phenyl-

[1] Parts per million.

acetic acid has also been tested in solution with cuttings of many plants at various seasons but its action is not sufficiently strong for it to be of value in plant propagation work. Only infrequently did phenylacetic acid cause rapid cell division in cuttings and then only at relatively high concentrations of 200 p.p.m. whereas indolylacetic often induces rapid cell division at 50 p.p.m.

Derivatives of histidine (*α-amino-β-glyoxaline-4*(*or* 5)*-propionic acid*).

Glyoxaline-4(or 5)-propionic acid has been used in five tests carried out at appropr seasons with *Abelia grandiflora*, *Buddleja alternifolia*, *Ledum palustre*, *Lonicera yunnanen* and *Osmanthus delavayi*; of these tests those with *Buddleja* and *Osmanthus* showed signs of weak activity of the substances when used at 50 p.p.m. The compound was not nearly active as indolylacetic acid.

Glyoxaline-4(or 5)-acetic acid was also tested, as the hydrochloride, and proved relati inactive; it was used in this form in a further six series of tests and was also applied in p form, without result, to rapidly growing young tomato plants.

II. Naphthalene derivatives

In attempting to find a compound of marked activity with a less toxic action than naphthylacetic acid closely related compounds have been tested. The standard substance these comparisons was α-naphthylacetic acid.

3 : 4-*Dihydro-1-naphthylacetic acid*

This compound has been tested in sixteen series using readily propagated plants such *Buddleja alternifolia*, *Lonicera yunnanensis* and *Tricuspidaria dependens*. Those offering s difficulty such as *Arctostaphylos patula*, *Ceanothus thyrsiflorus*, *Erica arborea*, and oth offering greater difficulty such as *Cephalotaxus drupacea*, *Picea pungens* and *Pittospo tenuifolium*. In general the results closely resembled those obtained with α-naphthylac acid, in all three aspects, namely, a higher percentage of cuttings rooted, more roots veloped on each cutting, and acceleration of the rooting process. This compound did induce roots in cuttings of plants which failed to respond to α-naphthylacetic; it offered material improvement over α-naphthylacetic acid, and was evaluated as an equally active useful compound. The sodium salt used at the same concentrations gave similar results

1 : 2 : 3 : 4-*Tetrahydro-1-naphthylacetic acid*

Tetrahydro-1-naphthylacetic acid was tested in fourteen series using readily propaga plants such as *Buddleja*, *Lonicera* and *Tricuspidaria*, less readily propagated species such *Ceanothus thyrsiflorus* and *Escallonia montevidensis*, and difficult species such as *Picea p gens*, *Pittosporum tenuifolium* and others including several conifers. Generally the res closely resembled those obtained with the preceding compound. This more fully satura compound was on the whole almost as active as the dihydronaphthylacetic acid when co pared at similar concentrations.

1 : 2 : 3 : 4-*Tetrahydronaphthylidene-1-acetic acid*

(*a*) In this compound the side chain is linked to the main cyclic group by a double bond. There are two stereo-isomerides (*cis* and *trans*) widely differing in melting-points, 92° C. (A) and 163° C. (B). The isomeride with the lower melting-point has been tested in thirty-two

series, involving approximately 10,000 cuttings. The species tested included readily and less readily rooted plants and difficult plants. This acid has proved active in the highest degree. Cell division was frequently stimulated and a rapidly formed thick zone of new tissue developed with many species, and root initiation followed. From the propagation point of view, as judged by the number of cuttings rooted satisfactorily, and by the subsequent development of these cuttings, this acid has proved to be of major importance. The results obtained by its use have been as good, or better, than those with any other chemical used at Wisley. A series of tests made with the sodium salts of this acid have shown it to be equally effective.

(*b*) The isomeride with the higher melting-point has been tested in eighteen series, and a further seven using the sodium salt. Briefly it is less active, and of less value. In its reactions it is less active than tetrahydronaphthylacetic acid and thus offers no improvement over α-naphthylacetic acid.

"*Mixed acids*"

The synthesis of these "mixed acids" involves the dehydration of tetralolacetic acid. The elements of water can be removed from this substance in two different ways, forming either dihydro-1-naphthylacetic or the tetrahydronaphthylidene-1-acetic acids. In practice a mixture is obtained of the approximate composition:

70 % dihydro-1-naphthylacetic acid,
20 % tetrahydronaphthylidene-1-acetic acid M.P. 92° C.,
10 % „ „ „ „ M.P. 163° C.

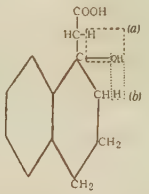

Fig. 1. The withdrawal of the elements of water from tetralolacetic acid to form: (*a*) tetrahydronaphthylidene-1-acetic acid, (*b*) dihydronaphthylacetic acid.

In seventeen tests of the acids and twelve tests of the sodium salts the mixture has proved to be highly active.

The tests have included a wide range of species from readily propagated plants such as *Calceolaria violacea*, *Fuchsia* (varieties), *Lonicera yunnanensis* and *Nuttallia cerasiformis*, to those more difficult such as *Elaeagnus pungens*, *Loropetalum chinense*, *Sarcococca hookeriana* and *Arbutus unedo*. In its action this mixture has given results very closely approximating to those obtained with the acid of highest activity, i.e. (A), the tetrahydronaphthylideneacetic of lower melting-point. This is, no doubt, due to the presence of 20 % of this compound, the 10 % of the acid of lower activity has not seriously impaired the action, whilst the 70 % of the dihydronaphthylacetic acid is also very useful. The sodium salts have given precisely similar results.

Sodium tetralolacetate

This compound from which the mixture of acids is formed has also been tested to see if it were possible to omit one stage in the synthesis. Nine series of tests have been carried out at different times of the year, using a selected range of appropriate species. The results show this substance to possess a fair degree of activity so that cuttings may be stimulated to root freely by its use, but the tests did not show it to be so highly active as to render the last stage in the synthesis unnecessary. The activity was of a lower order than that of tetrahydronaphthylideneacetic acid (M.P. 92° C.).

2-Phenanthrylacetic acid and 3-phenanthrylacetic acid

These two compounds have been tested separately on plants likely to show signs of any activity. The reactions obtained with *Buddleja alternifolia*, *Lonicera yunnanensis* and *Tricuspidaria dependens*, used at the appropriate seasons, show these compounds to possess a very low degree of activity, they are valueless for propagation.

Cyclo*Pentylideneacetic acid*

This compound was tested four times at different seasons of the year. It was found to be almost inactive as a root-producing substance when tested with responsive species.

Fluorenylideneacetic acid

A few tests made with this compound have shown it to be inactive in root initiation, neither did it cause cell division to take place rapidly when used at concentrations at which other substances proved active.

Fluorescent compounds

As claims have been made, but as yet largely unsubstantiated in this country, that the growth of plants may be increased by the application to soil or plant of certain dyestuffs derived from fluorescein and similar compounds, it seemed desirable to test certain of these fluorescent compounds as root-forming substances. The following have been found inactive in all the tests made with suitable species when tried at the same time as effective solutions (used at similar concentrations), viz.: diamino-acridine-di-ethyl-ω-sulphonate, pyranine (hydroxy-carboxy-fluorescein), fluorescein, diethoxy-dihydro-di-indole.

III. Tests of root-forming substances applied to entire plants

As claims have been made that minute quantities of growth substances may stimulate the growth of entire plants it is desirable that the experience gained at Wisley be placed on record.

A. Seeds—early growth

Exp. I. Soaking garden peas, 1936.

Method. The variety used was Gradus, and seed was selected by size and weight to give as uniform a sample as possible. The treatment used consisted in soaking counted seed at the rate of 100 seeds in 160 c.c. of dilute solutions of the growth substances for 24 hr. at 20° C. The concentrations used were 100 p.p.m. for indolylacetic acid, and α-naphthylacetic acid, and 300 p.p.m. for phenylacetic acid. After treatment the seeds were washed quickly in distilled water. A control series was soaked in distilled water at the same rate and temperature for the same period. A further control series of dry seed was used. Planting in boxes took place immediately after treatment, the seeds were placed in pure

washed sand at the same depth. Observations were made on the early stages of germination and growth, and an analysis was made of the data collected from dried seedlings weighed in batches of ten. In all there were 24 series of seedlings each of 100 seeds.

Results. (*a*) Treatment resulted in slightly delayed germination in all cases.

(*b*) The development of the plumule was particularly retarded as shown by the delayed straightening of the plumular hook and by the delayed elongation of the plumule as measured a week after planting: controls, $2{\cdot}40 \pm 0{\cdot}039$ cm.; treated with indolylacetic acid, $2{\cdot}07 \pm 0{\cdot}036$.

A smaller effect was produced by phenylacetic acid, but naphthylacetic acid produced a more marked effect.

(*c*) The number of roots formed was increased by treatment, but their length was reduced.

(*d*) The statistical analysis of the dry weights of seedlings one month after planting showed a significant but small change due to the treatments. The dry matter had been distributed in favour of the roots of the seedlings from treated seed; the shoots weighed less than did those of the control series. The differences in the total dry weights between control and treated were not significant, the treatments did not accelerate the process of accumulation of dry weight. All the seedlings then showed a slight increase over their seed weight.

(*e*) Soaking in distilled water caused a slight, but significant, acceleration of development of the seedlings. The cotyledons yielded their stored products more rapidly as judged by their loss in weight.

TABLE 1. *The fresh weights and dry matter content of the parts of pea seedlings raised from seed soaked in various solutions*

Treatment	Av. total wt. 10 plants, fresh g. (1)	% dry matter (2)	Av. wt. 10 plants, shoots fresh, g. (3)	% dry matter (4)	Av. wt. 10 plants, roots fresh, g. (5)	% dry matter (6)
(*a*) Phenylacetic	$25{\cdot}03 \pm 0{\cdot}04$	8·06	$13{\cdot}70 \pm 0{\cdot}01$	8·01	$7{\cdot}51 \pm 0{\cdot}005$	7·19
(*b*) Indolylacetic	$27{\cdot}03 \pm 0{\cdot}05$	7·91	$13{\cdot}42 \pm 0{\cdot}02$	7·62	$7{\cdot}64 \pm 0{\cdot}008$	7·80
(*c*) Naphthylacetic	$27{\cdot}70 \pm 0{\cdot}06$	7·63	$11{\cdot}06 \pm 0{\cdot}02$	7·91	$9{\cdot}20 \pm 0{\cdot}02$	7·61
(*d*) Control: water	$31{\cdot}05 \pm 0{\cdot}08$	7·00	$16{\cdot}60 \pm 0{\cdot}02$	7·45	$8{\cdot}32 \pm 0{\cdot}01$	5·6

Exp. II. Soaking Delphinium *seeds*, 1938.

Method. Seeds of hybrid origin were soaked in excess of indolylbutyric acid, or α-naphthylacetic acid, used at a concentration of 20 p.p.m. for 44 hr. at 15° C., in September. They were washed and sown in a sandy compost in small pots. Control series were soaked in distilled water for the same period at the same temperature. A further control series were sown direct in the soil. There were twelve series each of 100 seeds.

Results. Four weeks after sowing a slightly higher percentage of seedlings had germinated in the treated series; this difference was of doubtful statistical significance.

Exp. III. Soaking Delphinium *seeds*, 1939.

Method. The above experiment was repeated a year later using precisely similar methods, but using another larger fresh sample of seed.

Results. No significant difference in the rate of germination was caused by the treatments, and 6 weeks after sowing the seedlings of the series did not differ in size.

Exp. IV. Soaking lily seeds.

Method. Seed samples of *Lilium columbianum*, *L. formosanum*, *L. humboldtii* and *L. humboldtii* var. *magnificum*, and *L. washingtonianum* were used. The seeds were soaked at constant temperature (20° C.) in excess solution of α-naphthylacetic acid at 20 p.p.m. for 48 hr. Controls were soaked in water. Samples were then withdrawn, counted and placed under germination conditions in a "Copenhagen" germination tank.

Results. Under regulated conditions of moisture and temperature there was no significant difference in the rate of the subsequent germination of the treated and control series; immediate germination was not brought about by the treatments.

Exp. V. Soaking Lilium monadelphum *seeds.*

Method. An experiment was made with *Lilium monadelphum* var. *szovitsianum* using indolylbutyric acid and α-naphthylacetic acid at 20 p.p.m. for 48 hr. The seeds were sown in sandy compost in pots in early November.

Results. There was no advantage derived from the treatment; immediate germination did not take place.

Exp. VI. The use of dusts with seeds of peas.

Method. Powders containing organic compounds of mercury are available on the horticultural market, for dressing seeds with dust to prevent various fungal diseases, such as the bunt of wheat and seed-borne diseases of peas. To one such powder small quantities of the root-forming substances were added to give a mixture containing 10 p.p.m. of α-naphthylacetic acid, another mixture was made to contain 10 p.p.m. of tetrahydronaphthylidene-1-acetic acid. Such mixtures were used as seed dressings for garden peas which were sown in boxes placed in a cool greenhouse in April. The mercurial dressing was also tested and a control series of undressed seed used.

Results. The germination and establishment subsequently observed under the favourable conditions of the experiment were highly satisfactory in all the series. No advantage, as judged by the number of seedlings or by the vigour of their growth, occurred from the use of the dusts.

Exp. VII. The use of dusts with lily seeds.

Method. Seeds of *Lilium canadense* were shaken in envelopes with weighed quantities of talc powder containing indolylacetic acid. To other seeds dust was applied containing α-naphthylacetic acid at 10 p.p.m. and the mercurial dressing mentioned above; to other seeds dust was applied containing only the mercurial dressing. The seeds were subsequently sown in open sandy composts in pots placed in a cool greenhouse.

Results. All the treatments failed to cause immediate germination to take place, and no gain over the control series of undusted seeds was observed.

B. Older plants

Exp. VIII. The application of growth substances to Calendula.

Method. A large number of seedlings of *Calendula officinalis* var. *radio* were raised from seed sown in March and transferred to small pots. Selection was then made of a uniform batch of 2000 plants. To the sandy compost in which the plants were grown measured volumes of the solution containing the growth substance were added periodically. The following five substances were used: α-naphthylacetic acid, indolylacetic acid, tetrahydronaphthylidene-1-acetic acid M.P. 92° C., glyoxalineacetic acid (hydrochloride), dihydronaphthylacetic acid.

The concentrations used were 0, control or distilled water, and treatments at 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}. There were eighty plants in each series. To each pot was applied 20 c.c. on four occasions during the early period of growth, the first "dose" was given at the "four-leaf" stage in early April.

Results. The two stronger solutions showed toxic effects 3 weeks after the first application. It was later possible to discern toxic effects from the solution at 1 p.p.m. after four applications of the solution. There was no sign of any stimulation of growth at any time with any solution, and the plants grew until ripe seed was gathered from the controls. The controls were always the most vigorous plants. The solution at 10^{-7} (1 part in 10 millions) had little effect.

Exp. IX. The application of growth substances to Calendula.

Method. The entire experiment was repeated at a later date so that treatment began in May, and in this case the applications were continued for 8 weeks; at flowering time the dry weights of the plants were obtained.

Results. Despite the wide range of concentrations again employed no stimulation was observed. The toxic effects varied directly as the dosage of chemical applied. There was no statistically significant increase, due to any treatment, in the dry weight of the plants, obtained by weighing batches of twenty plants. The dry weights ran parallel to the size of the plants, and the data on analysis confirmed the toxic nature of the substances at the higher dosage.

Exp. X. The application of growth substances to Coreopsis grandiflora.

Method. The methods used were as in Exps. VIII and IX.

Results. A similar result to that given in the two preceding experiments was obtained with this plant.

Exp. XI. Application of growth substances to Lilium henryi *bulbs.*

Method. In all experiments made with lily bulbs, to ensure the maximum uniformity obtainable, clonal material was used. This experiment was divided into two halves, one in the sheltered frameyard where the bulbs were in pots, the other in the open ground where the bulbs were in special unglazed earthenware containers 3 ft. long × 6 in. diam. By sinking these pipes in the ground to soil level it was possible to keep the roots cool, and on withdrawing the pipes and displacing the soil to examine the roots developed under conditions permitting free downward growth, and to provide protection from slugs and other pests. There were twenty pots and twenty cylinders in each series. The compost used was: loam 2 parts, sand 1 part, leafmould 1 part. 0·1 g. of indolylacetic acid in lanolin paste at 1 % was applied to the base of the bulb when planted. The control series received the lanolin only. The bulbs were examined in the pots at the end of the first year and a further application of indolylacetic acid made after cleaning the base of the bulbs. The bulbs grown in the cylinders were examined 2 years after treatment. The heights of the plants were recorded throughout the growing seasons.

Results. The average height of the treated plants grown in pots was in 1937 reduced by some 10–15 %, the number of flowers was similarly reduced. On examining the bulbs in the pots after one season there was no apparent difference in number, length or general development of the stem roots, nor was any large difference seen in the number of basal roots (possibly the treatment caused a few more roots), but the average length of the basal roots was reduced 5–7 % by the treatment. The series of plants grown in cylinders showed a reduction of some 8 % in height due to the treatment.

In the second season a decrease in height was again observed in the series grown in pots and re-treated, but no difference was seen in the series grown in the cylinders treated a year previously. The difference observed in the heights of the plants grown in pots was accompanied by a proportional reduction in the number of flowers. In the second year a more serious reduction took place in the length of the basal roots, amounting to 33–40 % of the average length, but this was accompanied by an increase of 10–12 % in the number of basal roots.

On examination of the roots of the bulbs in the cylinders after a two-year period of undisturbed growth subsequent to treatment, the differences observed between the treated and control series were so small as to be of doubtful significance so that the plants had recovered from any check experienced in elongation of their roots; neither was there any lasting effect in regard to the number of roots formed.

Exp. XII. Application of growth substances to normal composts in which Lilium henryi *grew.*

Method. The plants were grown in pots containing a useful compost made up by mixing loam 2 parts, sand 1 part, well rotted leafmould 1 part. The bulbs planted in spring bore a poor root system; on three occasions at monthly intervals small quantities 0·01 g. of α-naphthylacetic acid was added to the soil in solution at a concentration of 100 p.p.m. by pouring on to the soil 100 c.c. Control series received distilled water only. Throughout the season measurements were made of the growth, and flower production was closely observed. The bulbs and the roots were examined in autumn. The experiment was carried out for two seasons. The plants received a top dressing of sand and leafmould when the stem roots formed.

Results. During the first season the average height of the treated plants was reduced by 20–25 %, and the number of flowers produced showed a proportional reduction. The stem roots appeared equal in number of whorls developed in the two series, but there was a very slight reduction in length caused by the treatments. The top dressing of sand and leafmould received only one dose of the chemical so that the stem roots escaped part of the treatment. There was an increase of 40 % in the number of basal roots developed, accompanied by a reduction of nearly 50 % in their length: these shorter roots were very much thicker. They bore secondary roots normally and small tertiary branches were also formed from which root hairs developed. It was considered doubtful whether the total area of roots from which root hairs developed was increased by the treatments, for the gain in number of roots developed was offset by the reduction in length which was accompanied by a reduced number of lateral roots.

The data collected during the second season confirmed the observations of the first season, but there was a smaller difference between the treated and control series in the number of roots; the control plants had steadily increased their number since planting whereas the treated plants did not form many in the second year. The effect produced was an acceleration of root formation.

Thus, using suitable composts and following the usual practice of top dressing the pots as the stem roots formed, no material advantage accrued from the treatments when judged by the development of

the stems, leaves, flowers and stem roots. The accelerated development of a greater number of roots was at least partially offset by the reduced length to which these roots developed in the treated series.

Exp. XIII. Application of growth substances to clay and sandy soils in which Lilium henryi *grew.*

Method. Bulbs selected by size and fresh weight were planted in heavy clay, containing only 1 % of organic matter, and placed in the cylindrical pipes previously mentioned; others were placed in an open sandy compost also almost devoid of organic matter, in similar pipes. In each soil were two series each of twenty plants, a control series receiving distilled water, and a treated series to which the growth substance α-naphthylacetic acid was added in solution. The rates of application were: 250 c.c. at 400 p.p.m. (0·1 g.) after planting, and 100 c.c. at 400 p.p.m. (0·04 g.) on four subsequent occasions. Thus each cylinder received a total of 0·26 g. in the first year. In the second year each cylinder received 0·01 g. on two occasions making a total of 0·28 g. in 2 years.

The heights of the plants were measured throughout the season and flower production observed. The cylinders were removed and emptied with great care so that root development might be gauged and measured after the second season. No top dressing was practised.

Results. The control plants were taller in the sandy soil where they grew very well and flowered more freely than those in clay. In the first season the growth of the stem in length was reduced approximately 20 % by the treatments applied to both soils. The reduction in height was again accompanied by a proportional decrease in flower production.

In the second season the plants responded favourably to the treatment in both soils by taller growth than the controls. In the clay series the difference amounted to 40 %, in the sandy soil to approximately 17 %. The number of flowers produced was similarly increased.

In the sandy soil a marked profusion of stem roots developed in the treated series, and this was no doubt related to, and at least partially the cause of, the better stems observed. The increase observed in the stem roots in the clay series was less strongly marked. In the sandy soil in the cylinders the average length of roots formed in both seasons was reduced by the treatment but their number was increased. In the clay soil the addition of the α-naphthylacetic acid increased the number of the roots as in the sandy soil, but it gave an unexpected result, it caused also a material increase in the length of the roots. The control series in clay had very short roots. Thus the root system was unquestionably superior to that seen in the control clay series. Careful scrutiny showed that this increase in number and length had been effected chiefly during the second year for the younger roots were longer and more numerous than their controls. The noticeably improved growth of the stems was correlated with this improved root system. In all series the bulbs in the clay were smaller than those in the sandy soil, but the treatment had been without much effect in regard to their number or size.

Thus, under these adverse conditions of the soil where little humus was present, and more especially where aeration was also inadequate in clay, the addition of the root-forming substance proved effective in stimulating the production of roots in the first but more especially in the second season; this was related to an increased development of the stems in the second season. In sandy soil the stem roots responded to treatment, in the clay the basal root system was materially improved.

Consideration of results

I. *Growth substances and amino acids*

As here shown indolylacetic and especially indolylbutyric acid are highly active compounds bearing a close relationship to tryptophane. Phenylacetic acid, although active, is not sufficiently so for propagation purposes, it bears to phenylalanine the relationship shown by indolylacetic acid to tryptophane, whilst another derivative β-phenylpropionic acid proved of low activity in these tests. Derivatives of tyrosine, namely β-*p*-hydroxyphenylpropionic acid and *p*-hydroxycinnamic acid, proved inactive, as did derivatives of histidine, namely, glyoxaline-4(or 5)-propionic acid and glyoxaline-4(or 5)-acetic acid. Whilst tryptophane and phenylalanine can be regarded as possible precursors of active growth substances no activity was shown by the parallel derivates of tyrosine and histidine in these tests.

Using other plants the evidence differs. Boysen Jensen (1932) found *Aspergillus niger* capable of producing growth substances, active on oats, when supplied with tyrosine, histi-

dine and phenylalanine but some other amino-acids were not effective sources. Ehrlich (1912) showed strains of yeast to be capable of performing similar reactions with tryptophane. With *Rhizopus* growing on peptone the mechanism appears to be one of oxidative deamination for the yield of growth substance, indolylacetic acid, varied with the aeration (Bonner, 1932; Thimann, 1935).

When applied to coleoptiles of oats tryptophane has only a delayed action; positive results have also been obtained in tests made with stems of peas. These facts support the evidence from fungal metabolism and suggest that similar oxidative deamination may take place in the higher plants. In such tissues the occurrence of growth substances considered to be auxin *a* and *b* has been shown to be widespread (Kogl, Haagen Smit and Erxleben), whether indolylacetic acid is also widespread yet remains doubtful, but tryptophane is known to occur in seedlings.

The mechanism of deamination in the tissues involves theories concerned with the formation of amino-acids and proteins. Chibnall (1939) considers the α-ketonic acids as highly likely precursors of amino-acids. Thimann & Dolk (1933) suggest that by bacterial action tryptophane passes to indolyl acid via indolylpyruvic acid as a half-way stage.

By reduction of the amino-acids, liberating ammonia, the corresponding fatty acids may theoretically be formed, and the reverse reaction has been suggested as a possible means of synthesis of amino-acids. Regarding this hypothesis Onslow (1931) remarks that certain acids such as succinic and glutaric acids are widely distributed in plants, whilst others such as propionic and β-phenylpropionic are probably to be found.

If proteins are formed, according to another hypothesis, by the condensation of large polypeptide complexes the simpler compounds, such as histidine, phenylalanine, tryptophane and tyrosine, would be regarded as stable products of hydrolysis. The deamination must be regarded as a reversible reaction whose equilibrium under normal conditions does not permit high concentrations to accumulate in the tissues of those substituted fatty acids proved active as growth substances.

Jordan Lloyd (1937), discussing the synthesis and structure of proteins, remarks that "it is strange that after all these years [Hopkins & Cole discovered tryptophane in 1901] no-one has yet discovered the biochemical functions of tryptophane". Recent research has put forward one such function, as a precursor of a highly active growth substance, indolylacetic acid.

II. *Other active compounds*

Two further substances are added to the list of active compounds, namely, the tetrahydronaphthylideneacetic acids. The importance of structural configuration is shown by the difference in activity of the two stereo-isomerides, stable at ordinary temperatures. Recently, Hubert & Rappaport (1939) showed that phenylenediamine possesses activity of a low degree and Stoutemyer (1939) considered naphthylacetamide a substance of much value for propagation purposes as it proved less toxic than α-naphthylacetic acid. As the list is rapidly enlarging it becomes increasingly difficult to see the determining criteria in the structure of the active compounds. Theories have been set up only to suffer some rapid modification. Zimmermann *et al.* (1933) found carbon monoxide capable of inducing the initiation and stimulation of roots, and that the effects of ethylene, acetylene and propylene resemble those of auxin. Crocker (1935) considered these gases as pre-eminently anaesthetics and stimulants of low toxicity.

As carbon monoxide is an oxygen acceptor, the unsaturated hydrocarbons, hydrogen acceptors, no doubt both influence the oxidation systems, but whether they influence the deamination process to liberate growth substances must remain very doubtful until further data be available. Crocker states that ethylene apparently depresses or increases the rate of respiration depending upon the concentration in the air surrounding the plant, and on the time and plant organ used.

III. *The cell reactions*

Macroscopically the responses evoked with cuttings, the greater number rooted, the more profuse root system and the acceleration of the process, closely resemble catalytic phenomena. Underlying these responses are the reactions of individual cells; when applied to parenchymatous cells of herbaceous tissue as, for example, the cortical cells of a young tomato, cell division frequently follows the rapid extension of the cells which may cause an increase in volume of some six or eight times the original volume in 8 hr. time. When woody cuttings are treated, the prominent feature of the response is the repeated cell division of the cambium and adjacent phloem cells. The enlargement of these meristematic cells was not readily seen as cell division starts rapidly, whether the walls of these young cells, without the deposition of any cellulose, so readily respond by stretching has not been determined (Frey Wyssling, 1936). The induced rate of cell division may be maintained for several weeks so that 20 or more divisions may take place (Tincker, 1937), so that a thick zone of parenchymatous tissue results. The activity of chemical compounds can readily be judged by such a reaction. The mechanism of the response shown as cell division as yet remains to be fully analysed (Went & Thimann, 1937); but the growth substance might act as a "drug", or highly effective irritant influencing the protoplasm, the reactions of which lead to cell division. Alternatively, the active compound may function as a catalyst facilitating at least one phase of cell division. This view would imply a quantitative relationship between the catalyst and the surface covered. The approximate relationship between the tissues and the effective "dosage" is of the order of 10^{-4}. It would also be necessary to assume that the catalyst is distributed in cell division for rapid and repeated divisions frequently take place once the process commences.

Although these growth substances inhibit the development of buds, especially at higher concentrations when used on cuttings, the new cells produced always develop into roots.

IV. *Growth of entire plants*

The Wisley experiments confirm the recent published findings of Templeman (1939) that application of the growth substances does not cause an increase in dry weight. No stimulation such as reported by Grace (1938) or Amlong & Naundorf (1938) was observed, but in planting bulbs such as *Lilium henryi* root development may, under adverse conditions, be accelerated and stimulated. In planting pecan trees, Romberg & Smith (1939) used growth substances to accelerate root formation. For other possible uses of these substances the reader is referred to Pearse's review (1939).

Summary

1. Tested on cuttings, indolylacetic acid and indolylbutyric acid are active growth substances. Several derivatives of tyrosine and histidine proved inactive, and from phenylalanine substances like phenylacetic and β-phenylpropionic acids possess low activity.

2. Tetrahydronaphthylideneacetic acid, M.P. 92° C., is highly active, its isomeride less so. By the dehydration of tetralolacetic acid a useful active mixture is obtained.

3. 2-Phenanthryl and 3-phenanthrylacetic acid proved inactive, as did also cyclopentylideneacetic and fluorenylideneacetic.

4. Growth substances when applied to seeds by soaking caused the resulting seedlings to be no heavier than those untreated. When applied to annuals at a wide range of concentration these substances produced no stimulus but only toxic effects at higher concentrations.

5. Applied to bulbs the growth substances checked stem elongation. In unusual circumstances the growth substances may cause accelerated development of roots from bulbs.

6. The relationship between certain amino-acids and some active growth substances is briefly discussed.

7. Cell division is a prominent feature of the response shown by cuttings.

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LIGHTNING INJURY TO POTATO TUBERS

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(With Plate 9)

In only one of a number of accounts describing the effect of lightning injury on the haulms of potato plants is mention made of an effect on the tubers (Schultz, 1907*a*), and in this the injury was not in the first instance ascribed to lightning. The possibility of lightning being the cause was suggested by Hiltner (1907), and was later considered by Schultz (1907*b*) to be correct. Schultz (1907*a*) merely stated that some of the tubers, which at the time were only about the size of peas, were found on being cut open to be affected with a disorganization (Zerstörung) of the tissues proceeding from within outwards. No details were given of any examination of the affected tubers, although a species of *Fusarium* was identified on the rotting haulms. It seems worth while, therefore, to record a case in which much greater damage was done to tubers in a potato field struck by lightning, the crop being almost mature at the time.

The case was reported by Mr J. O. Page, an Inspector of the Ministry of Agriculture and Fisheries. The lightning struck the ground in a crop of Majestic potatoes in Holbeach Marsh, Lincolnshire, on 21 August 1938. A hole about 9 in. deep was made in the soil, and many potatoes were thrown out on to the surface, a number having their rose ends shattered and blackened. Mr Page first saw the affected area 8 days after the lightning, and some of the shattered tubers had rotted. He stated also that other tubers appeared to be affected with Watery Wound Rot (vide infra, however).

As the tops were dying down, the affected area in the crop was not so marked as is described in accounts of lightning damage to younger, vigorously growing crops. Nevertheless, some of the same symptoms were observed, notably the flattening and splitting of the haulms as described by Orton (1921), Burr (1933), Weber (1931) and others. But in the Holbeach Marsh case Mr Page stated that "each shoot which showed this flattened appearance had badly shattered tubers below ground".

Some tubers dug out of the ground about 4 ft. from the hole made by the lightning were forwarded to the Plant Pathological Laboratory, and are shown in Pl. 9. Apart from those that had been almost "shattered" at one end (fig. 1), the most characteristic markings visible from the outside were depressed blackened areas of various shape and size, rather hollow within, over which the skin was still intact, or broken and torn by shrinkage (fig. 2). Such markings could quite conceivably be regarded as "burns", and people who saw the tubers within 1–2 hr. of the lightning flash informed Mr Page that the blackened marks were on the potatoes then.

Very frequently the markings were on two sides of the tubers, in a manner rather suggesting that the electric current may have passed through the tubers, causing burns only at the points of entry and exit. Mr Page suggested that passage of the current through the tubers would be very understandable as the soil was relatively dry at the time, and this would be the

path offering least resistance. Although in one or two cases when the tubers were cut open deep pits were found extending into the flesh from each side (fig. 4), in no instance were these pits actually found to meet, and they usually proved to be comparatively shallow. They varied from a few millimetres in depth, where the injury was only 1 cm. or so in diameter, to 1 cm. or more in depth under the larger wounds. Specimens examined about 3 weeks after the occurrence revealed the formation of a marked callus layer round the shallower pits. Within the latter were the rather shrivelled, greyish remains of dead cells and starch grains, sometimes with bacteria and fungus mycelium present, the rest of the cavity beneath the tightly stretched skin being empty. Even injuries as deep as 1 cm. were sometimes dry and punky beneath the skin, but in other cases bacterial and fungus rots had become established.

Two of the tubers sent to the laboratory when cut across were found to have the whole of the central tissue killed, still white in colour (rather as in tubers that have been frozen), but delimited from the healthy cortical cells by wider or narrower bands of lightly browned cells (figs. 5, 6). The whole effect was rather suggestive of Watery Wound Rot, but no mycelium was present in the affected tissue. Bacteria were present in the intercellular spaces in parts, but as it was already 10 days after the lightning flash it could not be stated with certainty whether these had any significance or not. It seems possible either that the central tissue in these tubers was killed by the electricity or else that the trouble was due to bacteria invading the pith from wounds. Of the two explanations it would seem most likely that the former was the true one, the effect resembling the killing of the pith in portions of the haulms without any of the leaves being injured, as described by Weber (1931) and others. It is rather difficult to understand, however, why the pith tissues should not have been similarly affected in tubers such as those shown in figs. 3 and 4. In any case, the condition called to mind Schultz's description of a "disorganization proceeding from within outwards", although there was no evidence in either case of there having been any progressive advance in the areas affected. These curious symptoms also render it somewhat doubtful whether the rot mentioned by Mr Page was actually Watery Wound Rot.

Summary

A description is given of injuries to tubers caused when lightning struck a potato field in Lincolnshire. The injuries included splitting, superficial and deep burns, and in some cases a general killing of the pith tissues.

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EXPLANATION OF PLATE 9

Royal Kidney potato tubers, injured by lightning at Holbeach Marsh, Lincolnshire, 21 August 1938, and photographed 10 days after the lightning had struck the crop.

Fig. 1. Tuber with partially shattered end, shown in section in fig. 3.

Fig. 2. Tuber with blackened, depressed areas suggestive of burns, over some of which the skin has broken. Shown in section in fig. 5.

Fig. 3. Section through the tuber shown in fig. 1.

Fig. 4. Section through a tuber showing pits suggestive of burns, extending towards one another from opposite sides.

Figs. 5, 6. Sections through tubers in which the whole of the central tissue was killed, considerable portions of the cortical tissues being still living.

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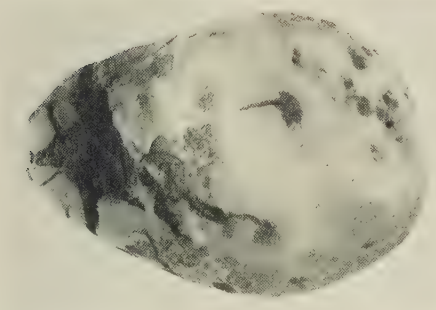

Fig. 1.

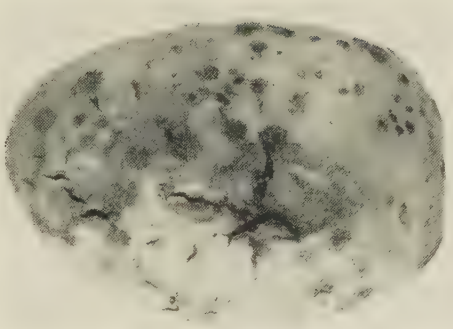

Fig. 2.

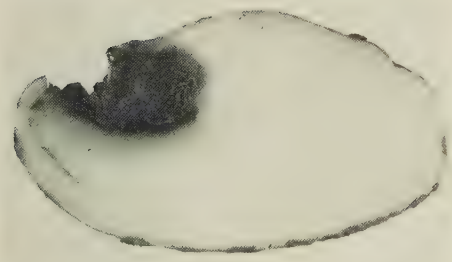

Fig. 3.

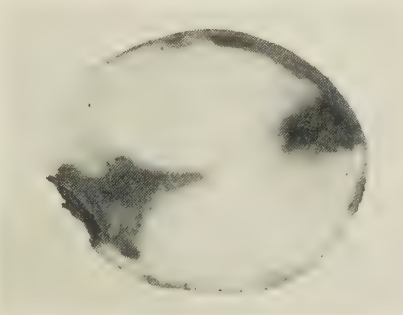

Fig. 4.

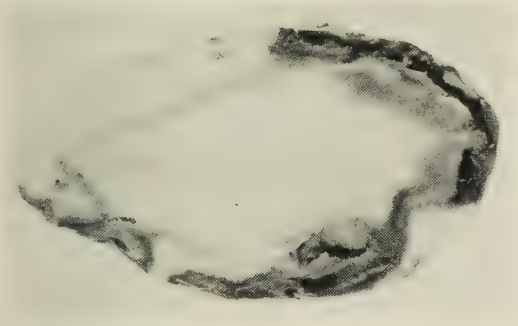

Fig. 5.

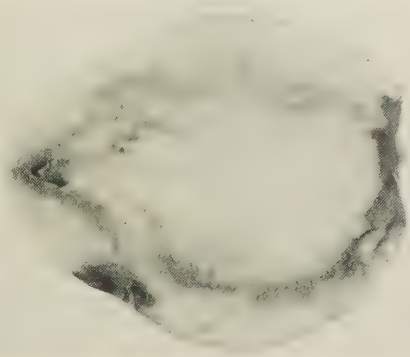

Fig. 6.

SAMUEL.—Lightning Injury to Potato Tubers (pp. 196–198)

SOIL CONDITIONS AND THE TAKE-ALL DISEASE OF WHEAT

V. FURTHER EXPERIMENTS ON THE SURVIVAL OF *OPHIOBOLUS GRAMINIS* IN INFECTED WHEAT STUBBLE BURIED IN THE SOIL

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Experiments on the survival of *Ophiobolus graminis* in artificially infected wheat straw buried in soil (Garrett, 1938) indicated that the fungus disappeared most quickly under those soil conditions, such as good aeration, adequate moisture and medium to high temperature, favouring general microbiological activity. Loss of viability was accelerated by addition to the soil of organic materials poor or lacking in nitrogen, such as rye-grass meal, starch and glucose, but retarded by addition of dried blood, containing some 13% nitrogen, or of ammonium carbonate. It was suggested that the "resting" mycelium of *Ophiobolus* is attacked and decomposed as a source of nitrogen by the other soil micro-organisms breaking down the straw, and that the provision of an alternative and more readily available source of nitrogen, such as dried blood, protected the *Ophiobolus* mycelium from decomposition.

Microscopical examination of infected straws from different experiments has since shown that a considerable further development of *Ophiobolus* mycelium takes place in the cells of infected straw after burial in soil. When straws are taken from the pure culture flask, they show comparatively little external mycelium, which is of the dark type, termed "macrohyphae" by Fellows (1928). The mycelium inside the straw is chiefly of the finer and hyaline type, the "microhyphae" of Fellows. When the straw is buried in soil, a development of the dark brown mycelium occurs inside the tissues, and it is markedly greater, and continues for longer, when excess of available nitrogen is present. Under soil conditions not exceptionally favourable to general microbiological activity, *Ophiobolus* may remain the dominant fungal colonist in the infected straw; in the latter stages of decomposition, its characteristic dark mycelium is often the only fungus visible. When *Ophiobolus* has died out from the majority of the infected straws, as shown by the wheat seedling test, there is often little but disintegrating mycelium to be seen.

These observations indicate that *Ophiobolus* mycelium can no longer be considered as passive material for decomposition by other soil micro-organisms. It is now suggested that its survival probably depends mainly upon the supply of *available* food material in the straw. Conditions favouring general microbiological activity in the soil would increase the rate at which the available food reserves in the straw are used up by *Ophiobolus* and associated micro-organisms. Available food material is limited by the supply of soluble nitrogen, since for the formation of fresh protoplasm one unit of nitrogen is required for about every 15 units of carbon assimilated. The effect of additional nitrogen in prolonging the viability of *Ophiobolus* in the infected straw is now most simply ascribed to its direct assimilation by the *Ophiobolus* mycelium.

On this new hypothesis, other micro-organisms must be assigned to a competitive rather than directly antagonistic relation towards *Ophiobolus*. The observed effect of adding rye-grass meal to the soil must be ascribed, firstly, to a rapid assimilation of the available nitrogen

in the soil surrounding the infected straws. Secondly, the total number of micro-organisms in the surrounding soil is greatly increased, and hence competition with *Ophiobolus* for available nutrients, especially nitrogen, in the infected straw itself is intensified.

Experimental

The technique employed in the following experiments has already been described (Garrett, 1938).

Exp. I. This experiment was designed to test the effect of varying the period of incubation of the straws, after inoculation with sand-cornmeal culture of *Ophiobolus*, in the culture flasks. Three periods of incubation were employed, 3, 7 and 12½ weeks, and the flasks of straw were kept in an incubator at 23° C. All were inoculated with *Ophiobolus* from the same series of sand + 3 % cornmeal cultures. The experiment was carried out in Harpenden allotment soil maintained at a moisture content of 50 % saturation throughout the experiment. Samples of two tumblers from each series, containing together 100 infected straws, were taken at 3-weekly intervals from 10 to 34 weeks. The results are given in Table 1.

Table 1. *Percentage of straws containing viable* Ophiobolus *mycelium*

	Weeks								
Series	10	13	16	19	22	25	28	31	34
No. 1, 3-weeks straws	98	87	72	62	59	94	94	45	80
No. 2, 7-weeks straws	98	97	97	98	84	99	86	91	98
No. 3, 12½-weeks straws	99	98	100	99	98	98	99	96	86

A sampling irregularity in the viability figures for the 3-weeks straws is absent from those for the other two series. Longer incubation of the straws in the culture flasks appears slightly to have increased the longevity of *Ophiobolus*. The longevity of the fungus in this experiment was remarkable when compared with that in previous experiments, and was tentatively attributed to the high nitrogen content (0·33 %) of the Harpenden allotment soil employed. Two more experiments were therefore conducted with this soil, as follows.

Exp. II. Six treatments of Harpenden allotment soil were employed in this experiment, and in all series the soil was maintained at a moisture content of 50 % saturation.

Series 1. Harpenden allotment soil, untreated. Total nitrogen 0·334 % and nitrate nitrogen 3·4 mg./100 g. soil, both on air-dry soil basis.

Series 2–4. With addition of calcium nitrate, ammonium sulphate and dried blood, respectively, at the rate of 12·5 mg. nitrogen/100 g. air-dry soil. This was equivalent to 0·55 g. nitrogen/100 g. air-dry straw, and to field dressings at the rate of 14 cwt. of calcium nitrate per acre, mixed with the top 10 in. of soil. Calcium carbonate was added to series 3 to neutralize the acidity produced by assimilation of the ammonia.

Series 5. With addition of 2·5 % of glucose.

Series 6. Allotment soil was previously mixed with excess of barley stubble, and kept in a shallow trench in the open for the first 3½ weeks of June. The straw and soil compost was kept moist and the surface layer cultivated at intervals. After this period, the soil was dried, and passed through the standard 1·8 mm. mesh sieve. By this treatment, a reduction was effected in total nitrogen content from 0·334 to 0·302 %, and in nitrate nitrogen from 3·4 to 2·1 mg./100 g. air-dry soil. The saturation capacity of the soil was altered by less than 1 % by the above treatment.

The results of this experiment are set out in Table 2.

The only treatment producing a clear-cut response in the experimental period was that of series 6. The effect of previous incubation of the soil with excess of cereal straw in reducing viability of *Ophiobolus* may tentatively be attributed to reduction in available nitrogen brought about by the treatment. The temporary increase in numbers of micro-organisms must also be taken into account, though the addition of 2·5 % of glucose (series 5) to this nitrogen-rich

soil exerted no obviously significant effect on the viability of *Ophiobolus*. Addition of 12·5 mg. nitrogen/100 g. soil in series 2–4 prolonged viability of *Ophiobolus* where ammonium sulphate and dried blood, respectively, were employed, but not where calcium nitrate was the source of nitrogen. Although nitrate nitrogen in the untreated soil of series 1 was, at the time of determination, only 3·4 mg./100 g. soil, the potential nitrogen-supplying power of the soil probably renders this figure of doubtful significance. Visible rate of decomposition of the infected straws, as judged by wastage, loss of coherence and rigidity, and darkening in colour, was as great in the untreated soil as in those with addition of 12·5 mg. nitrogen.

TABLE 2. *Percentage of straws containing viable* Ophiobolus *mycelium*

	Weeks				
Series	12	16	20	25	30
No. 1, untreated soil	100	100	100	91	77
No. 2, +12·5 mg. N as $Ca(NO_3)_2$	97	99	99	88	65
No. 3, +12·5 mg. N as $(NH_4)_2SO_4$	95	95	74	97	100
No. 4, +12·5 mg. N as dried blood	99	96	98	91	89
No. 5, +2·5 % glucose	97	95	92	87	71
No. 6, previously incubated with excess straw	98	91	71	60	46

Exp. III. In Exp. IV of a previous paper (Garrett, 1938) a remarkable acceleration in loss of viability of *Ophiobolus* was effected by crowding the infected straws closely together in the tumbler in one-quarter of the normal volume of soil. From this experiment, carried out with Slough loam, it was impossible to distinguish between the effects of increased soil aeration in the crowded straw series and reduction in available nitrogen owing to the increase in straw/soil ratio. It was decided to repeat the experiment in an elaborated form with Harpenden allotment soil, in which the effect of reducing the supply of nitrogen from the soil by the expedient of crowding the straws might be expected to show particularly well.

Series 1. The infected straws were crowded together at the rate of 100 straws (= 9 g. air-dry straw) to 100 g. (= 90 c.c.) of air-dry soil. When the straws had been loosely packed into the empty tumbler, 100 g. of soil was sufficient to fill up all interstices and provide for a thin covering of soil on top.

Series 2. The straws were packed at the normal rate of 50/200 g. of air-dry soil.

Series 3. As in series 1, but with addition of 1 % ammonium sulphate solution instead of water, to provide nitrogen for decomposition at the rate of 0·56 g. nitrogen/100 g. air-dry straw.

Series 4. As in series 2, but with addition of 0·25 % ammonium sulphate instead of water, to provide nitrogen at the rate of 0·56 g./100 g. air-dry straw.

The soil moisture content was maintained at 50 % saturation in all series. Results are given in Table 3.

TABLE 3. *Percentage of straws containing viable* Ophiobolus *mycelium*

	Weeks							
Series	9	12	15	18	21	24	27	30
No. 1, straws crowded, soil untreated	56	14	19	8	7	7	2	—
No. 2, straws normal packing, soil untreated	94	90	80	90	74	52	53	45*
No. 3, straws crowded, soil + N	46	49	44	9	6	11	9	—
No. 4, straws normal packing, soil + N	83	87	85	62	40	33	42	42

* Sample of one tumbler only.

The striking acceleration in loss of viability by *Ophiobolus* in series 1 and 3 as a result of crowding the straws indicates that increased soil aeration and reduction in available nitrogen are both important, the former being paramount. Addition of nitrogen to offset the effect of crowding in series 3 defers the final fall in viability to the 10 % level, but only for some

6 weeks. The addition of ammonium sulphate to the uncrowded straws in series 4 increased loss of viability by *Ophiobolus*. This discrepancy is unimportant, since this soil appears to contain sufficient natural reserves of nitrogen for decomposition of straw added at the normal rate, as indicated by the preceding experiment.

The differences in amount of visible decomposition in this experiment were of interest. Straws of series 1, crowded without additional nitrogen, were less decomposed than those of any other series. The earliest softening occurred in straws of series 3, crowded with nitrogen

Exp. IV. Since the total nitrogen content of Harpenden allotment soil was too high for any st response to added nitrogen to be expected, a typical arable soil from a field under normal rotati the Rothamsted Experimental Station farm was selected for this experiment. The soil was c characteristic Harpenden type, clay loam with flints, with a total nitrogen content of 0·20 %, nitrate nitrogen content of 0·3 mg./100 g. air-dry soil. Nitrogen as calcium nitrate was applie different series in amounts ranging from 1·56 to 12·5 mg. nitrogen 100 g. air-dry soil. This sc additions would correspond to a range of 1·75–14 cwt./acre mixed with the top 10 in. of soil. Sin straw to be infected had been cut into somewhat longer pieces than usual for this experimen weight of fifty straws was 5·3 g., so that the amount of nitrogen added per 100 g. air-dry straw from 0·06 to 0·47 g. The soil in all series was maintained at a moisture content of 50 % saturation. results of the experiment are given in Table 4.

TABLE 4. *Percentage of straws containing viable* Ophiobolus *mycelium*

	Weeks						
Series	7	10	13	16	19	22	25
No. 1, untreated soil	92	70	72	26	20	7	3
No. 2, +1·56 mg. N	89	82	82	57	37	18	8
No. 3, +3·125 mg. N	92	84	76	72	76	33	44
No. 4, +6·25 mg. N	91	86	84	82	73	46	38
No. 5, +12·5 mg. N	95	92	84	94	80	76	74

These results demonstrate a quantitative relationship between addition of nitrog calcium nitrate and survival of *Ophiobolus* in the infected straws. Even the smallest ad of nitrogen, that of 1·56 mg., produced an obviously significant increase in survi *Ophiobolus*. Macroscopical and microscopical observations on the infected straws fro different series demonstrated the relationship between addition of nitrogen and amo decomposition. Obvious decomposition of the straws, as judged by wastage, loss herence and rigidity, and darkening of colour, increased with the amount of nitrogen a to the soil. In series 4 and 5, considerable difficulty was experienced in manipulation straws for the wheat seedling test at the latter samplings.

Microscopically, the straws of series 5, with 12·5 mg. nitrogen, were characterize greater and more sustained development of the dark mycelium of *Ophiobolus* than w straws of series 1 in the untreated control soil. At the 16-week sampling, for instance majority of straws from series 1 the hyphae were mostly empty of protoplasm and ob disintegrating, whereas those in the straws from series 5 appeared to be in full vigour. microscopical observations, together with those on infected straws from other experiments, suggest strongly that nitrogen, whether as ammonium salt, as nitrate, or in organic form, is directly assimilated by the still active *Ophiobolus* mycelium in the infected straws.

Exp. V. This experiment was planned for a comparison of the survival of *Ophiobolus* in artificially *v.* naturally infected straws, and in laboratory tumblers *v.* small field plots. Two soils were chosen, Harpenden arable soil from the same site as that of Exp. IV, and a light textured sandy loam from

Bridgham, Norfolk, as used in Exp. III of a previous paper (Garrett, 1938). The latter soil contained 0·12 % total nitrogen, 7 % carbonates, and the *p*H value was 7·9. The Harpenden arable soil was known to permit only of a rather limited period of viability of *Ophiobolus* in the tumbler experiments; the Bridgham soil was selected on field evidence for survival of *Ophiobolus* for more than a year in this soil under clean, well-cultivated crops of sugar beet. The naturally infected straw was collected on 4 August 1938, from a crop of wheat on a chalk farm in Cambridgeshire. The basal part of the infected straw was cut off and trimmed of roots, and the pieces thus prepared stored cool and dry until the experiment was set up at the end of the month. The artificially infected straw was prepared in the usual manner. There was insufficient naturally infected material to permit of its use in the tumblers as well as in the field plots.

Series 1 *and* 2. Artificially infected straws buried in Harpenden and Bridgham soils, respectively, maintained at a moisture content of 50 % saturation in tumblers kept in the open laboratory; the temperature averaged 18° C. throughout the year. The normal complement of fifty straws was buried in each tumbler, which contained approximately 180 c.c. of soil.

Series 3 *and* 4. Artificially infected straws buried in Harpenden and Bridgham soils in small field plots. The soils were not air-dried, as for the tumblers, but were kept moist after collection from the field, and passed through a $\frac{1}{4}$ in. sieve before use. The 1200 infected straws of each series were mixed with 30 l. of moist soil, buried in a shallow pit, $4\frac{1}{2}$ in. deep, and covered with an additional 10 l. of the same soil. Each pit was topped after filling with a shallow layer of sand to mark out its position. After the taking of a bulk of soil sufficient to give the monthly sample of 100 straws, the cavity left was filled with sand, in order to avoid loss of time when taking further samples.

Series 5 *and* 6. As in series 3 and 4, but with naturally infected straws.

The experiment was started on 30 August 1938, and samples of 100 straws were taken from each series at monthly intervals to 9 months, after which a final sampling was made at 11 months. The sand-covered surface of the plots was kept free from weeds. The results of this experiment are set out in Table 5.

TABLE 5. *Percentage of straws containing viable* Ophiobolus *mycelium*

Series	Months									
	1 Sept.	2 Oct.	3 Nov.	4 Dec.	5 Jan.	6 Feb.	7 Mar.	8 Apr.	9 May	11 July
No. 1, Harpenden soil } Artificial straws,	100	65	22	12	3	0	0	—	—	—
No. 2, Bridgham soil } laboratory tumblers	100	100	90	58	40	18	2	—	—	—
No. 3, Harpenden soil } Artificial straws,	100	94	94	92	90	74	57	26	6	1
No. 4, Bridgham soil } field plots	100	100	100	100	99	100	100	97	76	23
No. 5, Harpenden soil } Natural straws,	81	83	79	83	71	60	46	46	28	13
No. 6, Bridgham soil } field plots	92	90	81	86	77	80	57	34	19	10

In series 1 and 2, with artificially infected straws in laboratory tumblers, the period to 50 % survival is approximately twice as long in the Bridgham soil ($4\frac{1}{2}$ months) as in the Harpenden soil ($2\frac{1}{3}$ months). In series 3 and 4 with artificially infected straws in the field plots, the period to 50 % survival is approximately 7 months in the Harpenden soil and 10 months in the Bridgham soil. The initial lag period of 5 months in the Harpenden soil and 8 months in the Bridgham soil, in which no appreciable loss of viability was recorded, may be ascribed chiefly to the low temperatures of winter. In series 5 and 6, with naturally infected straws, the distinction between Harpenden and Bridgham soil was not well marked; loss of viability by *Ophiobolus*, at first more rapid in the Harpenden soil, finally progressed farther in the Bridgham soil. It is difficult to explain this difference between naturally and artificially infected material, and further experiments with the natural material are required. The experiment has demonstrated, however, the ability of *Ophiobolus* to survive, in naturally infected straw as well as in the artificial product, in these two soils for 11 months.

Summary

Further experiments are reported on the survival of *O. graminis* in infected wheat straw buried in soil. The longevity of the fungus appears to be closely related to the available nitrogen content of the soil. Microscopical observation has shown that the characteristic dark mycelium of *Ophiobolus* slowly continues to develop in the artificially infected straw after this has been buried in the soil. This development of dark mycelium is greater, and also more sustained, in soils well supplied with nitrogen. Additional nitrogen is now considered directly to benefit *Ophiobolus*, by enabling the fungus to assimilate more of the undecomposed carbohydrate reserves of the straw. Conditions such as high aeration favouring general microbiological activity in the soil hasten the disappearance of *Ophiobolus*, probably by promoting a more rapid consumption of the available food material by the fungus itself and by associated micro-organisms; the supply of nitrogen normally limits both the amount of available food material and the survival of *Ophiobolus*.

I am indebted to the Department of Chemistry, Rothamsted Experimental Station, for the nitrogen determinations.

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EXPERIMENTS ON THE CONTROL OF BASAL ROT OF NARCISSUS BULBS CAUSED BY *FUSARIUM BULBIGENUM* CKE & MASS

WITH NOTES ON *BOTRYTIS NARCISSICOLA* KLEB

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THESE experiments, carried out during 1933–9, continue those of Gregory (1932) and Hawker (1935). Attention has been paid to the influence of environmental conditions (in the field and during storage of the bulbs) on the severity of the disease, and to the effects of certain fungicidal treatments, either when the bulbs are lifted or when they are subjected to the hot-water treatment.

Stocks of common varieties, including representatives of the trumpet (yellow and bicolor) Incomparabilis, Barrii, Leedsii and Poeticus sections, and totalling some 15,000 bulbs, were maintained at the Biological Field Station, Slough. They were divided into two approximately equal lots used for experiment in alternate years. Thus, one lot was lifted in July, used for experimental purposes during the storage period, and replanted in September or October. Notes were taken of the general appearance and rate of development of the foliage, date of flowering, number and quality of blooms, number of deformed blooms and of blind buds, rate of dying down of foliage, etc. The bulbs were lifted in the following July and the gain in weight determined. After storage the bulbs were planted as stock and formed material for experiment in the following summer (i.e. 2 years after the previous experiment).

Hawker (1935) pointed out that little rotting from *Fusarium* occurred in the stocks available, and suggested that storage conditions were unsuitable for the development of the disease. Stocks suspected of being infected with *F. bulbigenum* have been obtained from Holland, Cornwall, Lincolnshire and elsewhere, but their poor quality has usually been found to be due to other causes, viz. eelworms, mites, bulb-flies, *Botrytis narcissicola*, root-plate rot or stripe, or to a combination of these and *Fusarium bulbigenum*. These stocks have been freed from the three first-named pests by hot-water treatment, and in some cases were then found to be more or less infected with *Fusarium*. It is not improbable that *Fusarium* bulb rot, like soil-borne diseases in general, is accentuated by the frequent planting of susceptible hosts in the same plots. It is unfortunate that sporadic eelworm infections, unavoidably introduced with unhealthy stocks, made it necessary to replant in fresh ground from time to time.

While with a few exceptions losses from *Fusarium* have still not been heavy, the difficulty has to some extent now been overcome. Two stocks infected with *F. bulbigenum* but free from eelworm, viz. a large stock of Bicolor Victoria and a small quantity of van Waveren's Giant,[1] were obtained from Lincolnshire. In addition, certain stocks have been inoculated artificially by immersion in a suspension of *Fusarium* spores prior to planting. These in general behaved similarly to naturally infected stocks.

[1] The latter stock was obtained through the kindness of Mr E. R. Wallace of the Kirton Experimental Station.

Storage conditions

Rotting due to *F. bulbigenum* is favoured by high temperatures during storage of the bulbs. Gregory (1932) found that rotting of inoculated bulb scales was more rapid at 25° C. than at 17° C., and that losses in a naturally infected stock were about twice as great at 20–25 as at 10–12° C. He also showed that high storage temperatures were not in themselves injurious, since bulbs free from *Fusarium* developed normally after storage at 30° C. under conditions of good ventilation.

A more extensive examination of the effect of conditions of temperature, ventilation, etc., during storage has been carried out by the writer with both inoculated and naturally infected bulbs.

(*a*) *Temperature*

Bulbs of a number of varieties were either dipped in a cold suspension of *Fusarium* spores (marked with an asterisk in Table 1) or subjected to hot-water treatment with the addition of spores to the bath. They were then stored for 6 weeks under the conditions shown in Table 1. Losses were about ten times greater at the higher temperature (i.e. increased from 6·9 to 66·8 %).

Table 1. *Effect of storage temperature on losses from basal rot*

Variety	Date of treatment 1933	No. stored in each place	Temp. in shed ° C.	No. rotted in shed	No. rotted at 27° C.
Spring Glory (chips)	9 Oct.	75	0–17	0	50
,, ,, (large bulbs)	27 ,,	25	0–17	3	23
,, ,, ,, ,,	27 Nov.	25	0–17	13	25
,, ,, (chips)	27 ,,	25	0–17	0	23
,, ,, (large bulbs)	28 ,,	25	0–17	0	25
* ,, ,, ,, ,,	28 ,,	25	0–17	0	6
Victoria	11 Oct.	25	0–17	3	9
Golden Spur	29 Nov.	25	0–17	0	25
* ,, ,,	29 ,,	25	0–17	1	13
Henry Irving	11 Oct.	25	0–17	0	10
Glory of Leiden	27 Oct.	25	0–17	1	15
,, ,, ,,	28 Nov.	25	0–17	0	25
Incomparabilis (double variety)	1 Nov.	25	0–17	1	23
Barrii conspicuus	1 Nov.	25	0–17	1	24
,, ,,	28 ,,	25	0–17	2	23
	1934				
Spring Glory	17 July	25	13–30	9	15
,, ,,	7 Aug.	25	11–27	3	12
,, ,,	29 ,,	25	9–26	0	4
,, ,,	19 Sept.	50	4–22	0	20
,, ,,	20 ,,	25	4–22	5	18
,, ,,	7 Oct.	25	2–19	1	23
Incomparabilis (double variety)	20 Sept.	20	4–22	0	3
Total		620		43 (6·9 %)	414 (66·8 %)

Stocks of naturally infected bulbs or of bulbs which had been inoculated before planting (see above, p. 205) were stored from July to September in a cool cellar, where the temperature remained between 15 and 18° C., in an unheated shed where temperatures ranged from 12 to 26° C., reaching the higher levels only occasionally for a short period in the middle of the day, and at 27° C. All bulbs appeared to be sound when put into storage. The results (Table 2) again demonstrate the superiority of cool storage. The total percentage

losses from *Fusarium* in the cool cellar, in the shed and at 27° C. were 7·5, 12·4 and 22·7 respectively. Thus cool storage reduced the wastage of bulbs to about one-third of that occurring at the high temperature. Losses are particularly severe when bulbs are stored under warm conditions after hot-water treatment in the presence of spores. In view of this result Wood's (1936, 1939) suggestion that it may be desirable to "condition" bulbs by warm storage before and perhaps after hot-water treatment would be dangerous if applied to an infected stock.

TABLE 2. *Effect of storage temperature on losses from basal rot*

Variety	History	Period of storage	No. in each batch	No. rotted		
				Cellar	Shed	At 27° C.
Spring Glory	Inoc. *Fusarium* Sept. 1935	July–Sept. 1936	300	0	16	44
Henry Irving	Naturally infected eelworm and *Fusarium* 1934–5, hot-water treated 1935	,,	50	11	17	21
,, ,,	Naturally infected eelworm and *Fusarium* 1936–7, hot-water treated 1937	July–Sept. 1938	50	0	3	5
Victoria	Naturally infected *Fusarium*	,,	150	30	32	55
	Total		550	41 (7·5 %)	68 (12·4 %)	125 (22·7 %)

Gregory's statement that high temperatures are not injurious to healthy bulbs, provided that good ventilation is ensured during storage, has been in general confirmed. Healthy stocks stored at 27° C. remained sound but flowering was delayed and in some instances (particularly with Barrii and Leedsii varieties) the flowers were small and of poor quality, whilst those stored in the cool cellar flowered in advance of all others and gave blooms of good size and quality. This acceleration, which sometimes amounted to 7–10 days, was most marked with early flowering varieties and during cool weather when the flowers opened slowly. These results are in agreement with Dutch work, summarized by Purvis (1938). Cool storage, therefore, both reduces wastage from *Fusarium* and causes earlier flowering.

In contrast, bulbs infected with *Botrytis narcissicola*, in the writer's experience, have shown heavier losses at cool or moderate than at high temperatures. Certain stocks suspected by the growers of being infected with *Fusarium* were found (sometimes only after storage for several months) to be infected with *Botrytis narcissicola*. This was often associated with slight attack by eelworm or small bulb-fly. Three such stocks were used in storage experiments, and the results (Table 3) show that losses from *Botrytis* were greater at low temperatures.

TABLE 3. *Effect of storage temperature on losses from* Botrytis narcissicola

Variety	Period of storage	Storage temp. ° C.	No. in batch	No. rotted by *Botrytis* during storage
Poeticus Ornatus Maximus	Aug.–Oct. 1932	10–38	180	14
,, ,, ,,	,,	0–36	180	12
,, ,, ,,	,,	2–4	180	36
Poeticus Ornatus	July–Sept. 1935	27	100	15
,, ,,	,,	13–26	100	23
,, ,,	,,	15–18	100	27
Grandis	July–Sept. 1934	27	250	34
,,	,,	9–30	250	153
,,	,,	15–18	250	136
,,	,,	10	250	152

The results with var. Grandis were complicated by the fact that the stock was slightly infected with eelworm when purchased. This infection could not be detected by superficial examination, but in a few sample bulbs which were cut open traces of eelworm infection were found in the neck region. The great difference between the numbers of this stock rotting at 27° C. and at lower temperatures was partly due to a much slower development of the eelworm attack at the higher temperature. It may be noted that the survivors were hot-water treated before being planted and were free from both eelworm and *Botrytis* infection in the following season. It is not clear whether absence of attack by *Botrytis* was due to the actual killing of the fungus by the hot-water treatment or to the discarding of damaged bulbs before planting.

The effect of low temperature in aggravating attack by *Botrytis* and eelworm reduces to some extent the value of cool storage. In practice damage from eelworm would be controlled by the hot-water treatment. In the writer's experience *B. narcissicola* has only occurred in bulbs already attacked by eelworm or bulb-fly or in stocks which had been left in the ground for a number of years.

It should be possible to prevent unduly high storage temperatures by the use of the type of well-ventilated bulb store with an insulated roof recommended by the Ministry of Agriculture and Fisheries (1938) or of cool dry cellars. Where bulbs are stored in ordinary sheds or in warehouses the maintenance of suitable temperatures is often impracticable. Moreover, there is a danger of bulbs being subjected to excessive heat during transport with subsequent development of the disease in storage.

(*b*) *Ventilation and rate of drying of bulbs*

Over a number of years bulbs have been stored from July to September under conditions designed to accelerate or retard drying, viz. in relatively thin layers in trays, stacked to allow free circulation of air between them, or in closed boxes which were stacked closely together. Attention was also paid to the state of the bulbs when lifted, viz. dry and dusty or wet, and in the latter case whether or not much wet soil clung to the bases. The remaining details including temperature of storage are set out in Table 4.

Table 4 shows that rapid drying, especially immediately after lifting, diminishes wastage due to *Fusarium*. A batch of 900 Spring Glory bulbs, in lots of fifty, was included in the storage experiments under the variety of conditions indicated. These suffered no loss, showing that the conditions of storage were not in themselves harmful, a result which was confirmed by experiments with smaller batches of other varieties. The desirability of quick drying after hot-water treatment has been pointed out (Hawker, 1935).

The benefit of rapid drying at the beginning of the storage period is further illustrated in Table 5. The bulbs were stored either in the condition in which they were lifted or were given a preliminary cleaning whereby the bulk of the soil was removed. The latter treatment consistently gave a reduction in wastage.

The acceleration of drying due to early cleaning would be greatest with bulbs lifted from a wet sticky soil. Early cleaning gives advantage from the point of view of disease control, but there are two objections, viz. the pressing demands on labour at the lifting period, and the fear that a proportion of the bulbs may be damaged during the process. In the latter connexion it is relevant to record that none of the stocks of Table 5 showed any injury in the

following spring and that no damage resulted when roots of bulbs of vars. Golden Spur and Spring Glory were cut off near the basal plate soon after lifting.

TABLE 4. *Effect of ventilation during storage on losses from basal rot*

Variety	Condition when lifted	Storage conditions	Storage temp.	No. stored	No. rotten *Fusarium* in September
1. Grandis	Fairly dry soil, adhering to bases	Closed 14 days, then open	Unheated shed	200	32
,,	,,	Open 14 days then closed	,,	200	22
,,	,,	Open	,,	200	12
2. Victoria (naturally infected *Fusarium*)	Dry	Closed	27° C.	100	56
,,	,,	Open	27° C.	100	27
,,	,,	Closed	Unheated shed	100	28
,,	,,	Open	,,	100	20
3. Spring Glory (inoc. *Fusarium*)*	Fairly dry	Closed	27° C.	100	20
,,	,,	Open	27° C.	100	13
,,	,,	Closed	Unheated shed	100	7
,,	,,	Open	,,	100	5
4. Barrii conspicuus (inoc. *Fusarium*)*	Fairly dry	Closed	,,	50	15
,,	,,	Open	,,	50	3
5. Glory of Leiden	Fairly dry	Closed	27° C.	60	3
,,	,,	Open	27° C.	60	0
6. Glory of Leiden (inoc. *Fusarium*)*	Wet, soil adhering to bases	Closed	Unheated shed	50	10
,,	,,	Open	,,	50	4

* Inoculated before planting (see p. 205).

TABLE 5. *Effect of early cleaning of bulbs*

Variety	No. stored in each batch	No. rotting in storage	
		Stored as lifted	Partially cleaned when lifted
Victoria (naturally infected *Fusarium*)	100	23	16
Victoria (sound stock)	50	2	0
Glory of Leiden	50	6	2
Spring Glory	100	18	11
Golden Spur	100	3	0
Barrii conspicuus	50	5	3
Total	450	57	32

The foregoing experiments indicate that losses from *Fusarium* can be materially reduced by attention to conditions of storage. Bulbs should be dried as rapidly as possible after lifting and after hot-water treatment. In wet seasons rapid drying is facilitated by the removal of adhering soil by partial cleaning soon after lifting. When cleaned, bulbs should be stored under conditions of good ventilation and, if possible, at a temperature below 18° C.

Date of planting

Certain growers hold that early planting materially reduces the loss due to *F. bulbigenum*. This belief is based on the experience that considerable losses may occur among top-size bulbs stored for sale while smaller bulbs of the same stock which are planted give a good stand in the following spring.

Gregory (1932) planted batches of bulbs at intervals of about 2 weeks from September to November. With var. Poeticus Ornatus, the number of plants missing in the spring was much greater in those batches planted late in the autumn. Loss was not due to *Fusarium*, however, but to *Botrytis narcissicola*. With var. Victoria, the number of bulbs showing attack by *Fusarium* when planted was greater in batches planted in late autumn but the number missing in the spring showed no significant differences. In the earlier plantings rotting had obviously progressed in the soil, but in the later ones the numbers missing in the spring showed no increase over those already infected at planting time. Gregory suggested that the growers' experience might be explained partly by the development of other diseases, such as that due to *Botrytis narcissicola*, and partly by the possibility of greater liability to infection of the large bulbs stored for sale.

Similar experiments were carried out by the writer in which bulbs of vars. Poeticus Ornatus and Glory of Leiden were planted at monthly intervals from July to November. Stocks of these varieties were divided into batches of fifty bulbs and stored in an unheated shed until planted. The experiment with bulbs of var. Poeticus Ornatus gave similar results to those obtained by Gregory, i.e. the bulbs which were planted late gave a poor stand in the field due to attack by *B. narcissicola*. The last two batches of var. Glory of Leiden (planted 1 and 30 Nov.) contained respectively two and six bulbs partially rotted with *Fusarium*, and these were planted with the sound ones. No further rotting occurred in any batch after planting, so that a complete stand was given by all batches except the last two.

The beneficial effect of early planting, in reducing loss from *Fusarium*, may be ascribed to the fact that planted bulbs often experience lower temperatures in the late summer than bulbs which are kept in the warehouse. An experiment was carried out with var. Spring Glory to test this view. Batches of seventy-five bulbs were given the standard hot-water treatment (3 hr. at 43° C.), but with the addition of *Fusarium* spores to the bath, at intervals of approximately 3 weeks from mid-July to mid-October. Of each batch twenty-five bulbs were stored at 27° C., twenty-five in an unheated shed and the remaining twenty-five planted 24 hr. after treatment. Untreated bulbs were similarly stored and planted. The results are given in Table 6: the control batches are not included since they remained sound.

Table 6. *Effect of planting on rotting of inoculated bulbs*

Date of hot-water treatment	No. rotting in shed	No. rotting at 27° C.	Nos. rotting in planted lots			
			No. blanks in spring	No. rotten when lifted	No. rotting after lifting	Total losses
16 July	9	15	13	13	9	22
7 Aug.	3	12	2	3	9	12
28 Aug.	0	4	0	0	5	5
19 Sept.	5	18	0	0	3	3
17 Oct.	1	23	0	0	5	5

Losses of treated bulbs stored at 27° C. were considerable but were least in the batch treated on 28 August, i.e. at the period of maximum dormancy (Hawker, 1935). The batch treated in July and planted immediately, which was thus exposed to high soil temperatures after planting, showed serious blanks in the spring, and the same is true to a lesser extent in the batch planted on 7 August. This indicates that some bulbs had been attacked before they were able to begin growth. Other bulbs which were apparently sound when lifted were attacked by *Fusarium* during storage. In these the fungus had presumably become established on the surface of the bulbs (probably in the dead tissues at the base), but penetration of the living scales had not begun before the soil temperature became too low. With the return of warm conditions in storage during the following summer the fungus which had remained dormant at the base of the bulb was able to attack the living tissue. In batches treated at a later date, when the soil temperature was low, planted bulbs gave a full stand in the spring and were apparently sound when lifted. Nevertheless, a few bulbs in each lot were attacked during storage, indicating that even under cool soil conditions the fungus could establish itself at the base of the bulbs and survive until favourable conditions for attack occurred. As the untreated control bulbs showed no losses it is clear that infection was not already established at the time of treatment. Similar experiments on a smaller scale with other varieties gave confirmatory results.

Thus the growers' experience that early planting reduces losses from *Fusarium* may chiefly be due to the fact that soil temperatures at planting time (i.e. September) are usually too low for attack to take place, whereas bulbs stored in warehouses may still be exposed to relatively high temperatures. While bulbs should be planted by the end of September to give optimum results, very early planting of an infected stock, e.g. in July, has no advantages over storage under good conditions and cannot be recommended as a method of controlling the *Fusarium* disease.

Fungicidal treatments

While attention to conditions of storage would effect a material reduction in the losses caused by *F. bulbigenum* yet complete control cannot be obtained by these means.

Fungicidal treatment of bulbs to control *Fusarium* disease has been frequently advocated, the fungicide in general being added to the hot-water bath which is used as a routine treatment to control eelworm (van Slogteren, 1931; Weiss, 1929, 1930, 1932; McWhorter & Weiss 1932; Gregory, 1932; Wallace, 1934–5, 1936). This method has given promising results with a number of fungicides.

Hawker (1935) showed that the addition of 0·5% formalin to the bath had no harmful effect on growth or flowering and was in general beneficial. Little information was obtained on fungicidal effect since usually both control and treated bulbs remained sound, but infection readily took place when spores were added to the hot-water bath in the absence of any fungicide. Losses from this cause were at a minimum when bathing was carried out at the time of maximum dormancy of the bulbs.

Gregory (1932) suggested that it might be desirable to apply fungicidal treatment before the date when the hot-water treatment may safely be undertaken in order to prevent losses in the early part of the storage period. A series of trials designed to test the toxicity of certain fungicides to the fungus and to the bulbs themselves was carried out in the autumn so that his results probably bear no close relation to the effects of such treatments when applied

early in the storage period. McWhorter & Weiss (1932) claim striking results from the application of fungicides at lifting time under American conditions.

Experiments on fungicidal treatment of bulbs during the seasons 1934–9 have been concerned both with the addition of a fungicide to the hot-water bath and with various cold steeps and dry dustings applied at or soon after lifting time.

(*a*) *Incorporation of fungicides in the hot-water bath*

Stocks of a number of varieties were hot-water treated at various dates from July to September. Some of these were apparently sound, others were infected naturally with *F. bulbigenum*, others had been inoculated by soaking in spore suspension before planting, and yet others were more or less infected with eelworm. Damaged or obviously infected bulbs were in general discarded before treatment. In the majority of experiments formalin was added to the bath to give a 0·5 % solution; other batches of bulbs were hot-water treated in water alone and subsequently steeped for 5 min. either in cold water or in a cold solution of formalin (0·5 %). Uspulun (0·25 %) was also used in the bath in some experiments. After treatment the bulbs were stored at room temperature and examined in September, with the exception of a small lot of naturally infected bulbs of var. Van Waveren's Giant which was obtained and treated in September and examined after 6 weeks storage at 23° C.

(i) *Effect on* Fusarium *disease.*

Representative data relating to fungicidal effect are given in Table 7.

TABLE 7. *Effect of fungicides in association with hot-water treatment on subsequent losses from basal rot*

Variety	Condition when treated	Treatment	Date of treatment	No. treated	No. subsequently rotted in storage
Van Waveren's Giant	Naturally infected *Fusarium*	H.W.T.	27. ix. 38	20	16
,, ,,	,, ,,	H.W.T. (U.)	27. ix. 38	20	11
Spring Glory	Inoculated *Fusarium**	H.W.T.+C.W.	8. vii. 38	200	99
,, ,,	,, ,,	H.W.T.+C.F.	8. vii. 38	200	18
,, ,,	Sound	H.W.T.	31. viii. 38	40	6
,, ,,	,,	H.W.T. (F.)	31. viii. 38	40	2
,, ,,	,,	None	31. viii. 38	40	1
Henry Irving	Infected eelworm	H.W.T.	6. vii. 37	300	178
,, ,,	,, ,,	H.W.T. (F.)	8. vii. 37	300	126
,, ,,	,, ,,	H.W.T. (U.)	9. vii. 37	300	116

* Inoculated in previous autumn (see p. 205).

Treatment: hot water alone (H.W.T.) or with uspulun (U.) or formalin (F.) incorporated; or followed by steep in cold water (+C.W.) or cold formalin (+C.F.); further details in text.

These and eight similar experiments, involving a total of 6300 bulbs, consistently gave a reduction in the number of bulbs rotting after hot-water treatment when a fungicide was added to the bath or was applied subsequently as a cold steep. The number of bulbs rotting in some experiments was small but the results obtained were in agreement with those with more heavily infected stocks.

The results of all the experiments in this connexion are summarized in Table 8 where a distinction is made between bulbs infected with, and those free from, eelworm.

TABLE 8. *Effect of fungicides in association with the hot-water bath on bulbs infected with or free from eelworm*

Condition of bulbs	Treatment	% rotted
Free from eelworm	H.W.T.* without fungicide	36·1
,, ,,	H.W.T.* with fungicide	7·0
Infected with eelworm	H.W.T.* without fungicide	42·9
,, ,,	H.W.T.* with fungicide	28·2
Total bulbs	H.W.T.* without fungicide	41·3
,, ,,	H.W.T.* with fungicide	23·9

* Hot-water treatment (3 hr. at 43° C.).

The beneficial effect of the fungicide is much greater when the bulbs are free from eelworm. In stocks infected with eelworm the obviously soft bulbs were discarded before treatment, but fungal attack had commenced at the base of the scales in a proportion of the remaining bulbs which were not obviously soft. Hawker (1935) pointed out that when the fungus is established internally fungicidal treatment is unable to prevent further rotting of the bulb. Thus, a proportion of the bulbs in an eelworm infected stock cannot be saved by fungicidal treatment, and the beneficial result obtained in such a case is probably due to the prevention of the spread of viable *Fusarium* spores to sound bulbs during bathing.

Uspulun was about equally effective as formalin in reducing the number of bulbs rotting in the three experiments in which these fungicides were compared. Formalin, however, is comparatively cheap and is effective both when added to the bath and when used as a cold steep immediately after hot-water treatment.

(ii) *Effects on growth and flowering.*

Hawker (1935) showed that the addition of 0·1–1·5 % formalin to the hot-water bath had no harmful effects on foliage, date of flowering, or quality and number of blooms. Further work with healthy stocks has confirmed this result. Formalin (0·5 %) caused a definite acceleration of flowering in three out of ten experiments, the date of flowering in the remaining trials being uninfluenced.

The effect of uspulun in the bath was compared with that of formalin in five trials with healthy stocks. Flowering was retarded by uspulun in three of these, in one instance for 7 days. In the two experiments where flowering was not delayed by uspulun the blooms opened in a spell of warm sunny weather, which by hastening opening tended to mask any difference between treated and untreated lots.

Formalin applied as a cold steep following hot-water treatment induced slightly earlier flowering than in batches hot-water treated without fungicide in three out of ten trials, slightly later in three, and had no effect in the remainder. The amount of flower injury and the number of blind buds in eelworm infected stocks treated early in the storage season were increased by steeping in cold fungicide immediately after hot-water treatment. A similar amount of injury was caused by steeping in cold water, suggesting that the injury was due to the sudden change of temperature.

(iii) *Effect on increase in weight of bulbs during the growing season.*

Hawker (1935) recorded that in eleven out of sixteen trials hot-water treatment with the addition of formalin to the bath gave a greater increase in bulb weight during the growing season than was obtained by hot-water treatment alone. In twelve subsequent trials ten have

given a similar positive result the remaining two showing no difference. The addition of uspulun to the bath gave an increase in bulb weight during the growing season comparable with that provided by formalin.

In three out of four trials steeping the bulbs in cold formalin after hot-water treatment gave a slightly smaller increase in weight of bulbs than that in corresponding batches which were hot-water treated with or without the addition of formalin to the bath. A similar harmful effect was obtained by steeping the bulbs in cold water after hot-water treatment. Severe damage was obtained by Wood (1934–5) when bulbs were plunged into cold water immediately after hot-water treatment, but this damage was not consistently obtained. McWhorter & Weiss (1932), however, recommended that bulbs should be dipped in a cold solution of formalin, mercuric chloride, calomel (mercurous chloride) or ceresan after hot-water treatment, but suggested that it might be desirable to allow an interval of 2 hr. between removal from the bath and chemical treatment. Weiss (1932) gave figures showing a greater net increase in weight during the growing season when bulbs were dipped in a cold solution of ceresan or formalin after hot-water treatment than when the same chemicals were incorporated in the bath. Mercuric chloride gave a similar increase by either method of application. All fungicidal treatments gave significant increases in weight compared with hot-water treatment alone and the proportion of top-size bulbs was also increased. Weiss concluded that "successive treatment", i.e. the application of fungicides as a cold steep after hot-water treatment, was superior to "simultaneous treatment", i.e. the incorporation of fungicides in the bath.

The writer's experiments show neither the considerable gains from "successive treatment" obtained by Weiss nor the severe damage due to plunging in cold water described by Wood, but they suggest that with formalin this method may be less safe than the incorporation of the fungicide in the bath. It is established, however, that the addition of 0·5% formalin to the bath is harmless, that it materially reduces losses from *Fusarium* and that it may be beneficial to the bulbs irrespective of the presence of the fungus: it can, therefore, be recommended as a routine precaution.

(*b*) *Early treatment with cold fungicidal steeps and dusts*

Losses from *Fusarium* following hot-water treatment can be reduced materially by the addition of a fungicide to the bath, but with severely infected stocks considerable damage may occur before hot-water treatment can safely be carried out. Thus, a naturally infected stock of var. Victoria sustained severe losses every year and approximately half of these occurred before the normal date (late August) of hot-water treatment. A series of replicated experiments was carried out over a number of seasons to test the effect of treatment with cold fungicidal steeps or dusts applied at or soon after lifting time. Stocks of certain varieties were divided into batches of fifty or occasionally twenty-five bulbs and steeped in solutions of formalin (0·5, 1·0 and occasionally 5·0%), mercuric chloride (0·1%) or the proprietary mercurial fungicides uspulun (0·25%) and aretan (0·25%), or were dusted with the proprietary compounds ceresan, improved ceresan, folosan or brassisan which were usually diluted to one-fourth with talc. Control batches were usually of two kinds, viz. untreated bulbs and bulbs steeped in cold water alone. The effects of the treatments on losses in diseased stocks (either naturally infected or inoculated in the previous autumn) and on growth and flowering of healthy stocks were recorded.

(i) *Effect on* Fusarium *disease.*

The behaviour of four representative stocks is shown in Table 9 which gives the percentage loss in treated and untreated batches of a naturally infected stock, an inoculated stock and two relatively healthy stocks in which the losses were low.

TABLE 9. *Effect of early fungicidal treatments on losses from* F. bulbigenum

Treatment	Percentage loss due to *Fusarium*			
	Var. Victoria naturally infected	Var. Grandis inoculated in autumn	Var. Grandis relatively healthy	Var. Golden Spur relatively healthy
None	23	22	6·5	5
30 min. in cold water	18	28	15·5	—
,, cold 1·0 % sol. formalin	14	3	2·5	1
,, cold 0·25 % sol. uspulun	—	5	1·5	0·5
,, cold 0·25 % sol. aretan	—	—	0	—
Dusted brassisan	—	7*	—	0
,, folosan	—	—	—	0
,, ceresan	—	—	0·5	0

* Dust used undiluted, in all other instances dusts diluted to one-fourth with talc.

As a rule, soaking the bulbs in cold water led to increased losses: in eight trials, involving 625 bulbs, an average of 17·8 % rotted from *Fusarium* after being soaked for ½ hr., compared with 14·1 % in the untreated batches. When the bulbs are raised from sticky soil, however, washing may accelerate drying by removing adhering soil and thereby reduce infection.

Steeping the bulbs in a fungicidal solution reduced the losses below those in the untreated controls or in the batches soaked in cold water. Thus, in eleven trials fifty-three bulbs rotted out of 1175 soaked for ½-1 hr. in 0·5–1·0 % formalin, while 130 bulbs rotted in the control lots, i.e. 4.7 % compared with 11·1 %. Other fungicidal steeps gave a similar degree of control.

The dusting treatments were about equally as effective as the steeps in controlling the disease. They have the intrinsic merit that their use does not involve wetting the bulbs which in itself is known to increase wastage.

(ii) *Effect on rotting due to* B. narcissicola.

Three stocks which were apparently sound when treated were afterwards found to be infected with *B. narcissicola*. Soaking in cold water greatly increased the wastage, and steeping in cold formalin solutions was ineffective probably owing to the resistance of the sclerotia. The less volatile fungicides which leave a protective deposit might be more useful in this connexion since the development of mycelium from the sclerotia would be checked.

(iii) *Effects on growth and flowering.*

The relative safety of the treatments listed above (p. 214) was determined by application to healthy stocks. With two exceptions no treatment produced any adverse effect on rate of growth or the healthy appearance of the foliage. Bulbs dusted with improved ceresan were killed. Steeping in a cold 5·0 % solution of formalin caused severe damage to the basal rings which was visible before planting. A number of plants was missing in the Spring and the survivors flowered late and gave a small crop of inferior blooms. Gain in weight was

negligible or even negative. No injury was shown when bulbs were treated with a 1·0% solution of formalin.

The effects of these treatments on date of flowering are summarized in Table 10, the comparisons being between the treatments cited and untreated bulbs. A slight acceleration or delay means one of less than 2 days, a marked delay one of more than 2 days.

TABLE 10. *Summary of effects of treatments with cold fungicides on date of flowering*

Treatment	No. of trials				
	Total	Slight acceleration	No effect	Slight delay	Marked delay
Soaked in cold water	20	0	17	3	0
,, cold 0·5% formalin	15	0	10	5	0
,, cold 1–2% formalin	19	1	14	4	0
,, cold 0·25% uspulun	17	1	8	8	0
,, cold 0·5–1% uspulun	3	0	1	2	0
,, cold 0·25% aretan	7	1	5	0	1
,, cold 0·1% $HgCl_2$	13	0	0	1	12
Dusted* ceresan	7	0	3	4	0
,, folosan	7	0	6	1	0
,, brassisan	7	1	5	1	0

* All dusts diluted to one-fourth with talc.

Steeping the bulbs in a cold solution of mercuric chloride caused a marked delay. Certain organic mercurial fungicides (uspulun and ceresan) had a similar but less marked effect. The remaining treatments (with the exception of 5·0% formalin already discussed and not included in Table 10) did not cause any serious delay in flowering although slight delay was more frequent than acceleration.

(iv) *Effects on increase in weight of bulbs during the growing season.*

Steeping in a 5·0% solution of formalin injured the bulbs and seriously reduced the amount of gain in weight during the growing season. Steeping in a 0·1% solution of mercuric chloride increased the gain in weight compared with that of untreated bulbs. This treatment also delayed flowering (Table 10), so that it is possible that there is some correlation between late flowering and gain in weight. No other treatment influenced gain in weight to a noticeable extent. Thus, in ninety-six trials treatment with fungicides gave an increased gain in forty-nine cases and a decrease in gain in forty-seven when compared with untreated bulbs.

For some years difficulty has been experienced in obtaining stocks infected with *F. bulbigenum*, and Wallace (1936) reports that enquiries relative to the disease at the Kirton Experiment Station, Lincs, have recently fallen off considerably. It is not clear whether this is due to a succession of seasons unfavourable to the disease or to the general use of fungicides in the hot-water bath. One grower who regularly experienced heavy losses from *Fusarium* following hot-water treatment now reports that such losses are rare and attributes this improvement to the regular use of formalin in the hot-water bath. Should the disease reappear to a serious extent the early applications of a cold fungicidal steep or dust offers a promising method of control additional to the provision of suitable conditions of storage as discussed above.

Summary of results

1. Losses during storage in stocks inoculated or naturally infected with *Fusarium bulbigenum* were greater at 27° C. than at lower temperatures. Stocks infected with *Botrytis narcissicola* showed greater losses at the lower temperature.

2. Rapid drying of bulbs after lifting reduced wastage caused by *Fusarium*. Partial cleaning of bulbs soon after lifting accelerated drying and reduced losses. Bulbs were uninjured by the cleaning process.

3. Evidence suggests that the growers' view that early planting reduces rotting from *Fusarium* is due to the fact that soil temperatures in September may be lower than those obtaining in bulb warehouses.

4. The number of bulbs rotting after hot-water treatment against eelworm was reduced by the addition of formalin (0·5 %) or uspulun (0·25 %) to the bath or by dipping the bulbs in a cold solution of formalin (0·5 %) immediately after hot-water treatment. This reduction was most marked in stocks free from eelworm attack. None of the fungicidal treatments had any harmful effects on growth and flowering when bathing took place at the correct time (i.e. late August).

5. The use of certain cold fungicidal steeps and dusts soon after lifting also reduced wastage by *Fusarium*. Most of these treatments had no effect on growth or flowering.

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THE IDENTIFICATION OF CERTAIN VIRUSES FOUND INFECTING LEGUMINOUS PLANTS IN GREAT BRITAIN

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(With Plates 10 and 11)

In a survey of the virus diseases of leguminous plants in Great Britain, it was not found possible to relate the virus of lettuce mosaic, which Ainsworth & Ogilvie (1939) found able to infect both culinary and sweet peas, to any other virus but, in the course of examining about seventy-five samples of material, a number of different viruses were identified. These, with possibly one exception, appear to have been described in other parts of the world, but the following notes include several new records for this country.

Methods

The identification of a plant virus usually depends on the reactions of a range of test plants and on certain physico-chemical properties of the virus. With few exceptions, the viruses which affect legumes have comparatively short survival periods *in vitro* and are inactivated by heat between 55 and 60° C. These viruses are mostly aphis-borne and not very easily transmitted by mechanical means (although the addition of carborundum to the inoculum facilitates infection), and there is often considerable variation between the results of a few tests (see Murayama, 1938). For these reasons the physical properties are of secondary importance for diagnostic purposes, and even when accurately determined the physico-chemical properties are, as yet, rarely sufficient for the delimitation of a virus of this group. It would not, for example, be easy to identify with certainty a virus with the one studied by Murayama (1938) for which the symptoms on broad bean only are described.

In the present work approximate values for the resistance to heat and the longevity *in vitro* were determined by subjecting crude undiluted sap to temperatures differing by 5° C. for 10 min. periods and by testing at 24 hr. intervals unpreserved sap stored at laboratory temperature, and the following test plants were employed: pea (*Pisum sativum* L. var. The Lincoln); sweet pea (*Lathyrus odoratus* L.); French bean (*Phaseolus vulgaris* L. var. Stringless Green Refugee, American seed); broad bean (*Vicia faba* L. var. Harlington Windsor); red clover (*Trifolium pratense* L., strain 183, Welsh Plant Breeding Station); white clover (*T. repens* L., strain 200, Welsh Plant Breeding Station); lettuce (*Lactuca sativa* L. var. *capitata* Hort., var. Cheshunt Early Giant); tomato (*Lycopersicum esculentum* Mill. var. E.S. 1); tobacco (*Nicotiana tabacum* L. var. White Burley); *N. glutinosa* L.; and cucumber (*Cucumis sativus* L. var. Butcher's Disease Resister).

The literature on virus diseases of legumes is extensive and rather confused. In order not to add to the confusion and to avoid making a choice of the names suggested by other workers for certain of the viruses studied, and in the hope that the International Committee for the Description and Nomenclature of Plant Viruses will shortly publish its recommendations, the viruses are dealt with according to the diseases they cause.

Bean mosaic

Common bean mosaic has a geographical distribution coextensive with the cultivation of *Phaseolus vulgaris*. It was first recorded in England by Grainger (1929) and subsequently by Ogilvie & Hickman (1937) and others, but there appears to have been no attempt to ascertain whether the virus of this country is the same as that described as *bean virus* 1 by Pierce (1934) in America.

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P. vulgaris showed symptoms similar to those described by Pierce (1934) and Harrison (1935*a*) for the Stringless Green Refugee variety, and the virus proved to be seed transmitted ~~both~~ by this host ~~and *P. coccineus*~~. None of the other test plants could be infected, but although this is in agreement with Zaumeyer & Wade's (1935) results, both Reddick & Stewart (1919) and Pierce (1934) found broad bean to be susceptible. The virus survived heating to 55° C. or 24 hr. storage, but it was inactivated by heating to 60° C. or after 48 hr. *in vitro*, and therefore does not differ essentially from *bean virus* 1.

Outbreaks of mosaic generally, if not always, originate from infected seed. From 7 to 20% of the seedlings raised in the glasshouse from infected stocks of commercial seed have shown symptoms of mosaic. Bean mosaic frequently occurs on ~~runner beans (*P. coccineus*)~~ climbing varieties of *P. vulgaris* grown under glass, but on the popular Guernsey Runner variety damage is not usually very severe. Young plants are slightly stunted and the leaves show a well-developed mottle (Pl. 11, fig. 9), but the mottle becomes less evident on the upper parts of the plant and no injury to the pods has been noticed. This result is partly accounted for by varietal reaction, Guernsey Runner being more tolerant to mosaic than Tender and True ~~Runner~~ climbing beans or Stringless Green Refugee and Canadian Wonder French beans, and partly by the higher temperatures prevailing in glasshouses. [Harrison (1935*b*) showed that bean mosaic symptoms become masked at a temperature of 30° C.] Ogilivie & Hickman (1938) recorded more severe damage to outdoor beans in the west of England. Evidence regarding the susceptibility of *P. coccineus* is inclusive

Enation mosaic of pea

Enation mosaic of *Pisum sativum* was first described in the U.S.A. by Osborne (1935) and the same disease was studied by Pierce (1935), Stubbs (1937) (who designated the virus as *pea virus* 1), and Johnson & Jones (1937), but there appear to be no records from other parts of the world.

A disease which agrees very closely with enation mosaic commonly occurs on sweet peas in south-eastern England. It was, however, only detected on culinary peas in one locality in Hertfordshire. The disease is characterized by the enations which develop on the undersides of the leaves of infected pea, sweet pea and broad bean.

The first symptom in pea is a prominent clearing of the veins 5–8 days after inoculation and, subsequently, there develops a yellow mottle which is liable to be mistaken for that of a type of yellow mosaic (see Stubbs, 1937, p. 246, Fig. 1). Several weeks after inoculation the mottle on the upper leaves is usually less pronounced, but well-developed enations arise on the lower leaf surfaces (Pl. 10, fig. 4). The size of the enations appears to be correlated with the growth rate of the plant (cf. Ainsworth, 1937), and this is probably the reason for the larger enations obtained experimentally on the variety Alderman (a climber) than on the Lincoln variety which is a dwarf. The Perfection variety was also susceptible. The pods are distorted to a greater or less extent (Pl. 10, fig. 5).

In sweet pea the first symptom is also a prominent yellowing of the veins, but this is followed by a rather characteristic yellow flecking of the leaves, enations from their under surface, and slight deformation of the pods. The flowers are not broken, but infection with enation mosaic has occasionally been associated with a few small rough lesions on the petals.

This virus causes a yellow flecked mottle in broad bean (Osborne, 1935, p. 163, Fig. 1A) and enations which are less conspicuous than those on pea and sweet pea.

Phaseolus vulgaris, clovers, lettuce, cucumber and the solanaceous test plants could not be infected.

The virus survived 50° C. or 24 hr. ageing but was inactivated at 55° C. or after 48 hr. storage.

Osborne (1938) found that repeated transference of enation mosaic virus through broad bean was associated with "attenuation" of the virus, but, in the present study, a strain of the virus kept for nearly 2 years on peas and sweet peas, and only transferred by mechanical methods, retained its virulence. Although 80–100% infection of test plants was not uncommonly obtained there were on occasion unaccountably low percentages of infection, and this increased the difficulty of determining the properties and host range of the virus.

Pea mosaic

Viruses able to cause mosaic symptoms in culinary and sweet peas, broad bean, and red clover have been described from all parts of the world (for literature see Stubbs, 1937). Most of these viruses, which show minor differences, are probably variants of one type, but their nomenclature is very confused, different strains having been named *pea mosaic virus* 1 (Zaumeyer & Wade, 1935), *pea virus* 2 (Stubbs, 1937), and *pea virus* 3 (Pierce, 1935). Of the mosaic viruses isolated from pea, sweet pea, broad bean, and red clover from numerous localities in Great Britain two types were selected for more detailed study.

The first strain is widespread on peas and broad bean. In sweet pea it gives rise to an almost imperceptible leaf mottle of light and dark green but causes severe flower break in coloured varieties. In pea the mottle, which is preceded by a vein-clearing stage, is rather more pronounced (Pl. 10, fig. 2) and affected plants are slightly stunted but the pods are normal in appearance. The Perfection variety (American seed) was immune but Veitch's Perfection (English seed) proved susceptible. This strain was transmitted to broad bean but not to any other of the test plants, although a very similar disease was produced in pea and sweet pea by a virus obtained from naturally infected red clover (Pl. 10, fig. 8).

The second strain has only been found naturally in pea in which it causes a pronounced mottle of the yellow type and stunts the plant. Perfection pea is immune while sweet pea and broad bean are susceptible and show a similar but more pronounced mottle to that caused by the first strain. Red clover was also successfully infected on several occasions (Pl. 10, fig. 8) and the virus returned again to peas. No other test plants could be infected.

No special study of the insect vectors was undertaken but the yellow strain was transmitted to both pea and sweet pea by bean aphides (*Aphis rumicis* L.) collected from naturally infected pea plants.

The mild mosaic virus survived 50° C. or 24 hr. storage but was inactivated at 55° C. or after 48 hr. *in vitro*. The yellow strain behaved in a similar way, but on one occasion it was not completely inactivated at 60° C. Further evidence that the two strains are closely related was obtained by protective inoculation experiments (p. 223).

The mild strain agrees fairly well with the mild mosaic of Stubbs (1937) caused by *pea virus* 2A, which also failed to infect red clover, while the second strain shows similarities to *pea virus* 3 (Pierce, 1935; Murphy & Pierce, 1937).

Sweet-pea streak

Streak disease of sweet pea has been observed in England for many years (Taubenhaus, 1914), and its etiology has been a subject for conjecture. Massee at first (1909) considered streak to be a physiological disorder, but later (1912) he attributed the disease to *Thielavia* [*Thielaviopsis*] *basicola*, and although Chittenden (1912) also found this fungus associated with streak he was unable to reproduce streak symptoms experimentally. Manns & Taubenhaus (1912) claimed that sweet-pea streak was caused by *Bacillus lathyri*, a claim which was supported by Paine & Bewley (1923). Ainsworth & Ogilvie (1939) found that the virus of lettuce mosaic was one agent of naturally occurring sweet-pea streak.

In America the general opinion appears to have been that sweet-pea streak was a bacterial disease, until recently when it was attributed to tomato spotted wilt virus (Snyder & Thomas, 1936) and to viruses from white and sweet clovers (Zaumeyer & Wade, 1933).

Several years ago when investigating the streak disease of tomato, bacteria, which in cultural characters resembled *B. lathyri*, isolated from both tomato and sweet pea proved non-pathogenic. During the present survey streak symptoms in all samples of sweet pea examined have been associated with virus infection, and it has been concluded that sweet-pea streak is, at the present time, a complex of virus diseases. It is, however, probable that the brown lesions which sometimes occur towards the base of the stem within 6 in. of the soil are due to fungal infection.

(i) *Sweet-pea streak virus*

One form of sweet-pea streak is characterized by streak lesions on the stems and petioles, a definite mosaic mottling of the leaves and by breaking of the flowers. These symptoms are the result of infection by a single virus to which pea, broad bean, French bean, and white clover are also susceptible. Attempts to infect other test plants failed.

The first symptom shown by sweet pea is a well-defined clearing of the veins of the younger leaves 5–10 days after inoculation. Later, a distinct but not very severe mottle of the leaves develops, brown necrotic streaks appear on the stem and petioles, and coloured flowers break in a manner indistinguishable from that of pea mosaic. At times the disease becomes more aggressive and seedlings are killed.

In *Pisum sativum* the first symptom is a vein clearing similar to that in sweet pea. Subsequently, the whole upper part of a young plant may become chlorotic, there is marked stunting, and occasionally the plant is killed. Usually, a yellowish mottle accompanied by necrosis of the veins develops and the plant is not killed. The pods on infected plants are smaller than normal, slightly deformed and exhibit dark necrotic spots (Pl. 10, fig. 6). The Perfection variety of pea proved to be resistant.

The first symptom in broad bean is reddish brown local lesions. Later, there is a slight vein clearing of the youngest leaves which is accompanied by the sudden appearance of circular necrotic spots on many of the older leaves. Dark reddish brown streaks develop in the stem (Pl. 11, fig. 12) and the plant is greatly stunted. The fruits observed on infected plants have been normal.

In French bean a few pale green spots about 2 mm. diameter, each surrounded by a fine necrotic line, sometimes develop on the inoculated leaves, but usually the first symptom is a number of small yellow coloured spots on the first and second trifoliate leaves (the first two

simple leaves having been inoculated) (Pl. 11, fig. 10). The leaflets of these leaves turn downwards (Pl. 11, fig. 11). Later, a more general veinal chlorosis develops, growth of the plant almost ceases and death usually results within 4–6 weeks.

White clover (*Trifolium repens*) responds with a mild mosaic mottling which is at times almost masked, but the virulence of the virus to peas and beans was in no way reduced by passage through this host.

The resistance to heat and the longevity *in vitro* of the sweet-pea streak virus were the same as for the mild strain of pea mosaic described above.

Although alfalfa mosaic viruses (Zaumeyer, 1938; Pierce, 1934, 1935), *white clover virus* 1 (Pierce, 1934; Zaumeyer & Wade, 1935, 1936), *pea streak virus* 1 (Zaumeyer 1938) and *Pisum virus* 3 (Chamberlain, 1939) are each able to produce symptoms in one or more hosts rather similar to the sweet-pea streak virus it is clear that none is very closely related to the sweet-pea virus. *Bean virus* 2 (Pierce, 1934; Zaumeyer & Wade, 1935 (*pea virus* 2)), white sweet-clover mosaic virus (Zaumeyer & Wade, 1935, 1936) and the severe pea mosaic virus of Johnson & Jones (1937), however, do appear to have a closer affinity to the sweet-pea streak virus. Both *bean virus* 2 and the white sweet-clover virus cause the characteristic drooping of bean leaves and are able to cause a mosaic disease in pea but they are apparently less virulent than the sweet-pea virus. Perfection pea is resistant to *bean virus* 2 but susceptible to the viruses of white sweet clover and severe pea mosaics. Red clover is susceptible to both *bean virus* 2 and white sweet-clover mosaic virus and white clover is resistant, but both hosts were susceptible to Johnson & Jones virus. After a study of the published descriptions of these three viruses it has been concluded that while the sweet-pea streak virus cannot with certainty be identified with any one of them the four viruses belong to the same group. Pierce (1934) drew attention to the similarities between bean viruses 1 and 2, but in view of the fact that pea mosaic virus affords partial protection against the sweet-pea streak virus, while *bean virus* 1 does not, it is possible that the sweet-pea virus is more closely related to the pea mosaic viruses. A definite decision on this point cannot be made without further experimental work.

(ii) *Lettuce mosaic virus*

No symptoms develop in sweet pea until 14–21 days after inoculation with lettuce mosaic virus when small brown lesions are seen on the main stem, usually just below the point of insertion of a leaf. These lesions may increase in size when death of the shoot may result. Sometimes the new shoots appear normal, sometimes they finally become streaked. No definite leaf symptoms have been observed, but the virus has been shown to be present in all parts of the shoots: the flowers are not broken. In one planting of sweet peas, where lettuce mosaic streak was prevalent, many of the streaked plants exhibited broken flowers, and this symptom was proved by analysis to be due to the plants being also infected with mild pea mosaic virus.

No necrotic symptoms are exhibited by *Pisum sativum*. After a temporary vein clearing a mild and rather blotchy mottle develops (Pl. 10, fig. 1). Infected plants are not noticeably stunted and produce normal pods. Perfection pea is susceptible.

Attempts to infect other test plants failed.

Lettuce mosaic virus survives heating at 55° C. or 24 hr. (occasionally 48 hr.) storage, but it is inactivated at 60° C. or by 72 hr. storage (Ainsworth & Ogilvie, 1939).

(iii) *Cucumber virus* 1

In 1938, from one locality in Hertfordshire, sweet pea plants showed streak symptoms and the causal virus proved to be of the *cucumber virus* 1 type. When inoculated into sweet pea seedlings severe streak symptoms developed after about 14 days, and death of the plants resulted. The virus was not studied intensively but it was shown to infect cucumber, tobacco, and *Nicotiana glutinosa* in which it caused symptoms indistinguishable from those produced by the type virus. Lettuce and pea could not be infected. In one experiment, streak symptoms developed in sweet pea seedlings inoculated with the virus from sweet pea, whereas the plants in a parallel series inoculated with the type *cucumber virus* 1 from cucumber remained healthy.

Viruses of the *cucumber virus* 1 type have been recorded by Whipple & Walker (1938) in America causing a streak disease in culinary peas, but no similar disease has been seen in this country.

During the last few years several cases of the disease of garden lupins (*Lupinus polyphyllus* Lindl.), caused by *cucumber virus* 1 (see K. M. Smith, 1936), have been recorded. Affected plants are stunted, the leaves chlorotic and variously mottled and, in addition, there are often dark purplish brown streaks on the stems and petioles. No attempt was made to transmit this virus to peas or other leguminous plants.

(iv) *Tomato spotted wilt virus*

Snyder & Thomas (1936) described a streak disease of sweet pea in California caused by the virus of tomato spotted wilt, and Whipple (1936) recorded a streak disease of pea caused by this virus. Although tomato spotted wilt is widely distributed in Great Britain, and inoculation experiments have shown both pea and sweet pea to be susceptible to the virus from tomato, no instance of natural infection of either of these hosts was recorded until April 1939 when a single sweet pea plant received for examination proved to be attacked by spotted wilt. This plant, which had been grown under glass, did not exhibit streak symptoms but it was stunted and showed yellow spots or broad streaks on the leaves similar to the leaf symptoms figured by Snyder & Thomas (1936, p. 258, Fig. 1 A). Identical symptoms were produced in young sweet pea plants by inoculation with spotted wilt virus from tomato, but, as reported by Snyder & Thomas, the virus was found not infrequently to remain localized in the inoculated leaves.

The first symptom in pea is the development of characteristic local lesions showing concentric rings (Pl. 10, fig. 7) and these were often the only symptom. When systemic infection occurred, spots rather similar to the local lesions developed on leaflets in the upper parts of the plant, the leaflets on one side only of a leaf sometimes being affected, and purplish superficial streaks developed on the stem. The systemically infected plants produced no fruits; death resulted. The susceptibility of other leguminous plants was not determined.

Protective inoculation experiments

Peas infected with the mild strain of pea mosaic virus could not be infected with the yellow strain, but a plant showing symptoms of enation mosaic was susceptible to either of these strains. The latter result is in accord with field observations which showed sweet peas to be commonly infected with both enation mosaic and common mosaic viruses. Double

infection with these viruses could on occasion be diagnosed in the field, when a sweet pea plant showed enations on the leaves and also broken flowers. Sweet pea plants infected with lettuce mosaic could be infected with any of the above viruses or with the sweet-pea streak virus. Bean mosaic virus did not protect French beans against the sweet-pea streak virus, but there was a delay of about 7 days in the appearance of symptoms due to the latter virus in pea plants previously inoculated with either the mild or yellow strains of pea mosaic.

No experiments were made with tomato spotted wilt virus or the virus of the *cucumber virus* 1 type from sweet pea.

Other diseases recorded

Of several other diseases suspected to be caused by viruses which were observed two may be mentioned. The first was a mosaic disease of white clover possibly identical with that caused by white *clover virus* 1 (Pierce, 1934) in America, but attempts to infect white clover and other plants were unsuccessful. The second was a disease of red clover which, as regards the symptoms in red clover, appeared to be the same as the vein mosaic described by Osborne (1937). This disease was kept under observation for nearly 2 years but the virus could not be transmitted by mechanical methods.

Table 1. *Summary of the susceptibilities of certain of the test plants to the viruses studied*

Virus of...	Bean mosaic	Mild pea mosaic	Yellow pea mosaic	Sweet pea streak	Enation pea mosaic	Lettuce mosaic	Cucumber virus 1 type	Tomato spotted wilt
French bean	+	−	−	+	−	−	.	.
Pea:								
var. The Lincoln	−	+	+	+	+	+	+	+
var. Perfection	−	−	−	−	+	+	.	.
Sweet pea	−	+	+	+	+	+	+	+
Red clover	−	−	+	−	−	−	.	.
White clover	−	−	−	+	−	−	.	.
Lettuce	−	−	−	−	−	+	−	+
Tomato	−	−	−	−	−	−	+	+
Cucumber	−	−	−	−	−	−	+	−

+ Susceptible; − immune; . no test made.

Summary

A survey of the virus diseases affecting leguminous plants in Great Britain was made in order to acertain whether the virus of lettuce mosaic, to which pea and sweet pea are susceptible, could be related to any other virus able to infect legumes. In the course of the work the viruses, which are briefly described and discussed in this paper, were recognized, but lettuce mosaic virus could not be equated with any one of them.

Sweet-pea streak disease was found to be invariably associated with virus infection and is, it is thought, a complex of virus diseases.

The susceptibility of a range of host plants to the viruses of bean mosaic, mild pea mosaic, yellow pea mosaic, enation mosaic of pea, lettuce mosaic and tomato spotted wilt, a virus able to cause sweet-pea streak, and a strain of *cucumber virus* 1 from sweet pea are indicated in Table 1.

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EXPLANATION OF PLATES 10 AND 11

PLATE 10

Fig. 1. Pea (var. Lincoln) showing symptoms caused by lettuce mosaic virus.

Fig. 2. Pea (var. Lincoln) showing symptoms of mild pea mosaic. (Healthy leaf on left.)

Fig. 3. Pea (var. Lincoln) showing symptoms of yellow pea mosaic.

Fig. 4. Under surface of leaf from pea (var. Lincoln) affected by enation mosaic.

Fig. 5. Pea pods (var. Lincoln) deformed by enation mosaic.

Fig. 6. Pea pods (var. Lincoln) spotted by the sweet-pea streak virus.

Fig. 7. Local lesions caused by tomato spotted wilt virus in pea (var. Lincoln). (Healthy leaflet on left.)

Fig. 8. Red clover leaflets showing symptoms of yellow pea mosaic virus (left) and red clover mosaic virus (right). (Healthy leaflet in centre.)

PLATE 11

Fig. 9. Guernsey runner bean showing symptoms of mosaic. (Healthy plant on left.)

Fig. 10. Stringless Green Refugee bean leaflets showing symptoms caused by the sweet pea-streak virus 15 days after inoculation.

Fig. 11. Stringless Green Refugee beans showing symptoms due to common bean mosaic virus (left) and sweet-pea streak virus (right) 15 days after inoculation. (Four weeks later the two right-hand plants were dead.)

Fig. 12. Broad bean 15 days after inoculation with the sweet-pea streak virus.

Fig. 13. Sweet peas 21 days after inoculation with the sweet-pea streak virus.

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Fig. 1.

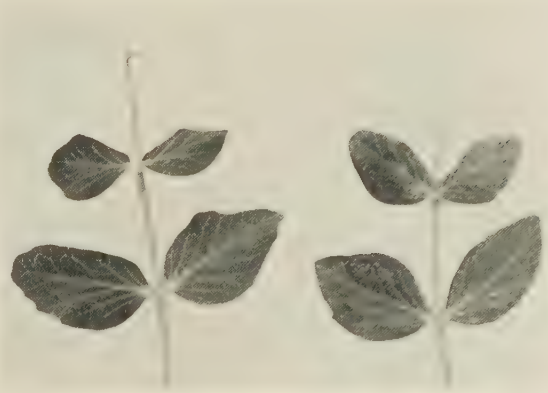


Fig. 2.

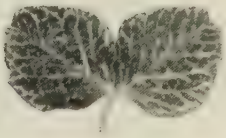


Fig. 3.

Fig. 5.

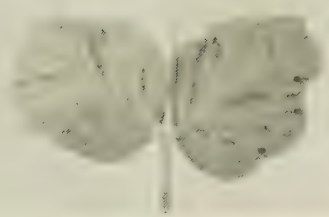
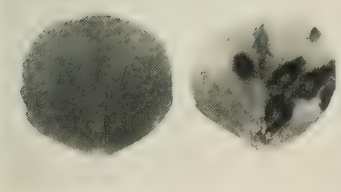


Fig. 4.

Fig. 6.



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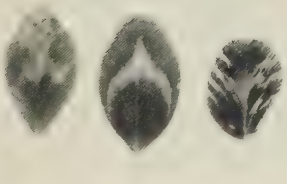

Fig. 8.

AINSWORTH.—The Identification of Certain Viruses found Infecting Leguminous Plants in Great Britain (pp. 218–226)

Fig. 9.

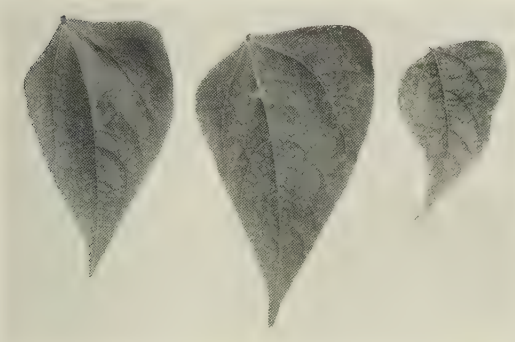

Fig. 10.

Fig. 11.

Fig. 12.

Fig. 13.

AINSWORTH.—The Identification of Certain Viruses found Infecting Leguminous Plants in Great Britain (pp. 218-226)

EVIDENCE AGAINST THE HYPOTHESIS THAT CERTAIN PLANT VIRUSES ARE TRANSMITTED MECHANICALLY BY APHIDES

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THERE appear to be two main types of insect-transmitted viruses: (*a*) persistent viruses (Watson & Roberts, 1939), whose vectors remain infective for an indefinite and often long period, and frequently exhibit an "incubation period" between infection feeding and successful transmission: (*b*) non-persistent viruses, whose vectors are capable of causing infection immediately after infection feeding, and have an infective period of short but comparatively constant duration.

It has generally been accepted that the persistent viruses have an obligate and complex relationship with their vectors, and that the non-persistent viruses are transmitted by "a purely mechanical process" (Hoggan, 1933).

The hypothesis of simple mechanical transmission of the non-persistent viruses rests on two arguments. The first assumes that the time taken for the virus to be transferred from the source of infection to the healthy plant is too short for it to have entered the body of the vector and been delivered *via* the blood stream and salivary ejaculation. There is no evidence as to the rate of circulation of the virus in such insects, or that this is the only route by which virus could be imbibed by them and returned to the plant. The second argument rests on the statements of various workers that when transmitting non-persistent viruses aphides are only capable of infecting a single healthy plant after a single infection feeding, or on very rare occasions, a second plant. This was taken to mean that the contaminated stylets were "cleaned" by feeding on the first plants and the insects thus mechanically rendered non-infective. The fact that the aphides retained their infectivity longer when fasting than when feeding was explained on the assumption that the stylets were not "cleaned" and the virus remained active "for approximately the same period as that during which the expressed juices of the plants remained infective" (Doolittle & Walker, 1928, referring to the transmission of Cucumber virus 1). We pointed out (1939) that, whether fasting or feeding, the retention of infectivity by vectors of non-persistent viruses is far less than would be expected from the inactivation rates of the viruses *in vitro*.

We (1939) therefore suggested a different hypothesis to account for such results, which more readily explains these anomalies. We suggest that the mechanism of transmission may not differ fundamentally between the persistent and the non-persistent viruses though there may be some method by which the non-persistent and some of the persistent viruses are short-circuited and returned more rapidly to the healthy plants. We believe the main difference between them to be that the non-persistent viruses are inactivated by a secretion produced by the aphides (and probably by other insects) when feeding, and that the persistent viruses are resistant to it. We postulate a close specific relationship between the non-persistent viruses (which resemble each other closely in their physical and other properties) and insects with a particular type of physiology. The Aphididae are closely related insects

with almost identical feeding habits, and there is no difficulty in supposing that many species of aphides would have similar relationships with similar viruses. Where persistent viruses, which are automatically assumed to have specialized relationships with their vectors, are transmitted by aphides, several can be carried by more than one species; this is also recorded for leaf-hopper transmitted viruses.

If the mechanism of transmission is similar for the two types of virus their behaviour should be similar in all circumstances not determined by the rate of inactivation of the viruses. A feature so characteristic of the persistent group of viruses that Storey (1939) has used it as a means of distinguishing between the two types of virus, is that the vectors are capable of transmitting infection to many healthy plants once they have become adequately infective. Once the non-persistent viruses have been inactivated by the vectors they cannot be transmitted to a succession of plants, but we have shown that fasting aphides develop a high degree of infectivity immediately after a short infection feeding. If the mechanism of transmission is similar for persistent and non-persistent viruses, then during this period of high infectivity the aphides should be capable of infecting as many healthy plants as they can feed on adequately. The results given in the present paper show that the viruses which we have tested can, in these circumstances, be transmitted to a number of healthy plants by individuals of *Myzus persicae* which have had access only once to a source of infection.

Methods

The methods used for culturing and handling the aphides were described by Watson (1936). Only two or three individuals were fed at one time and they were watched through a hand lens until the feeding position was assumed. The feeding period was timed from the assumption of the feeding position, if the aphid did not remove its stylets during this time.

The aphides were made to fast in a moist atmosphere for several hours. Each was given 2 min. feeding on the infected plants and then transferred to a succession of healthy seedlings. Most of the aphides fed soon after being placed on the leaves, the average time taken for a series of ten feedings being 35–50 min. The first three feedings were usually accomplished in 9–12 min.

Results

The results are given in Tables 1–3; those insects which either failed to give any infections or infected the first plant only of the series on which they were placed are not included. Such insects were in the minority, especially in the earlier experiments. The best results were obtained in April and May. During June–August infectivity was greatly reduced, and fewer consecutive infections were obtained. Watson (1936) recorded that the efficiency of *M. persicae* in transmitting Hy 3 virus is reduced during the summer months.

The only difference between the two sets of results given in Table 1 is that in series 1 the aphides were fed on six consecutive plants and in series 2 on ten. The number of plants in the series was increased because it was observed that the number of infections obtained on the last plants of the six in series 1 was greater than on the fifth plants, indicating that some of the aphides had not reached the limit of their capacity to infect although they had missed some of the later plants in the series. When the number of successive healthy plants was increased to ten, there was still a slight increase for the last plant of the series. It is probable that a few aphides would have infected later plants, but it is also possible that towards the

TABLE 1. *Consecutive infections obtained after a single 2 min. feeding of starved* M. persicae *on Hy 3 infected leaves. Time allowed on each healthy plant 2 min. The aphides which infected none, or only the first healthy plant are not included in the tables.*

Series 1. Six consecutive healthy plants. Out of thirty aphides three failed to give infection and eight infected the first healthy plant only.

		Consecutive healthy plants						
Date	Aphis no.	A	B	C	D	E	F	Total
7 Apr.	2	+	+	o	o	o	o	2
	3	+	+	o	+	o	+	4
	5	+	o	o	+	o	o	2
	6	+	o	+	+	o	o	3
	8	+	+	o	o	o	o	2
14 Apr.	11	+	o	+	o	o	o	2
	13	+	+	o	o	o	o	2
	14	o	+	o	o	+	o	2
	15	+	+	o	o	o	o	2
	17	+	+	o	o	o	o	2
	18	+	+	+	+	+	o	5
21 Apr.	24	+	+	+	o	o	o	3
	26	+	+	+	o	o	+	4
	27	+	o	+	+	o	+	4
	28	+	o	o	+	o	o	2
	29	o	+	+	o	o	+	3
	Total	14	11	7	6	2	4	

Series 2. Ten consecutive healthy plants. Out of thirty-six aphides eight failed to give infection, and eight infected the first plant only.

		Consecutive healthy plants										
Date	Aphis no.	A	B	C	D	E	F	G	H	I	J	Total
25 Apr.	1	o	o	+	+	o	o	+	o	o	+	4
	2	+	+	o	o	o	o	o	o	o	o	2
	5	+	+	o	o	+	+	o	o	o	+	5
	6	o	o	o	o	+	o	o	o	o	o	1
	9	+	+	+	+	+	+	o	o	+	o	7
5 May	12	o	+	+	o	o	o	+	o	o	+	4
	13	+	o	o	+	o	o	o	o	o	o	2
	15	+	+	+	+	o	+	o	o	o	o	5
	16	o	o	+	o	+	o	o	o	o	o	2
9 May	18	+	+	+	+	o	o	o	o	o	o	4
	19	+	+	+	+	o	+	o	o	o	o	5
	21	+	+	o	o	+	o	o	o	o	o	3
	24	+	o	o	o	+	o	o	o	o	o	2
	25	o	+	o	o	+	+	+	o	o	o	4
16 May	28	+	+	o	o	o	o	o	o	o	o	2
	30	+	o	+	+	o	o	o	o	o	o	3
	35	+	o	+	o	o	o	o	o	o	o	2
	36	+	o	o	o	+	o	o	o	o	o	2
	37	+	+	+	+	o	o	o	o	o	+	5
	38	+	+	o	+	o	+	o	+	o	o	5
	Total	15	12	10	9	8	6	3	1	1	4	

end of their active condition the aphides require longer periods on the healthy plant to cause infection, and that the 24 hr. allowed on the final plant of each set gave them an increased chance of becoming infected. Aphides frequently failed to infect two or three plants in the middle of a series but infected later plants, and occasionally the first or second plant was missed by an aphis which was highly successful at later feedings. Table 1 is not unlike some of those given for consecutive infections by the vectors of persistent viruses, e.g. Pea mosaic virus 3 (Osborn, 1935) and Curly-top virus of sugar beet (Freitag, 1936). The time taken for the individual feedings was much smaller, but the results for the persistent viruses show that the insects have the same tendency to miss larger numbers of plants as their total infectivity decreases. Freitag suggests that this is due to gradual exhaustion of the reserves of virus accumulated by the insects when feeding on the infected plant, so that at the later feedings there is less and less possibility of an infective dose being produced. This seems to be a very probable explanation for the decrease in infectivity of the vectors of both persistent and non-persistent viruses.

TABLE 2. *Consecutive infections obtained with* M. persicae *transmitting potato virus* Y. *Treatments as in Table* 1. *Out of twenty-four aphides four infected none, and three infected the first plant only*

		Consecutive healthy plants								
Date	Aphis no.	A	B	C	D	E	F	G	H	Total
23 May	1	+	+	o	o	o	o	o	o	2
	2	+	+	+	+	o	o	+	o	5
	3	+	+	o	o	o	o	o	o	2
	6	+	+	o	o	o	o	o	o	2
	7	+	o	o	+	o	o	o	o	2
	9	+	o	o	o	+	o	o	o	2
	10	+	o	+	o	+	o	o	o	3
	12	+	+	+	o	+	o	o	o	4
9 June	13	o	o	o	+	o	o	o	+	2
	15	+	o	o	+	o	o	o	o	2
	16	o	+	o	o	o	o	o	o	1
	17	o	+	+	+	o	+	o	o	4
	18	o	+	o	o	o	o	o	o	1
	19	o	+	+	o	o	o	+	+	4
	21	+	+	o	+	o	o	o	o	3
	24	o	+	+	o	o	o	o	o	2
	Total	10	11	6	6	3	1	2	2	

TABLE 3. *Consecutive infections obtained by* M. persicae *when transmitting Severe Etch virus. Out of eighteen aphides five gave none, and two infected the first plant only*

		Consecutive healthy plants								
Date	Aphis no.	A	B	C	D	E	F	G	H	Total
13 June	3	+	+	o	o	o	o	o	o	2
	5	+	+	+	o	o	o	o	o	3
	6	o	+	+	+	+	o	o	o	4
	9	o	+	+	o	o	o	o	o	2
21 June	11	o	o	+	o	o	o	o	o	1
	13	+	o	+	o	o	o	o	o	2
	15	o	+	o	o	+	o	o	o	2
	17	+	+	o	o	o	o	o	o	2
	18	+	+	o	o	o	o	o	o	2
	Total	5	7	5	1	2	o	o	o	

Table 2 gives a similar set of results for consecutive infection of healthy tobacco plants by Potato virus *Y* transmitted by *M. persicae*. They are very much the same as the Hy 3 results except that the insects were not quite so successful on the later plants of the series. There seems to have been a tendency in the June part of the experiment for the aphides to miss the first plant, and infect the later ones, but it is not possible to determine from the data whether this is a special property of the virus, or merely an effect of external conditions which would be the same for all the viruses.

Table 3 shows a few consecutive infection results for Severe Etch virus, also transmitted by *M. persicae*. The virus was kindly supplied by Mr B. Kassanis, who has studied it in considerable detail, and finds that in most of its properties it resembles Hy 3 and the other non-persistent viruses (Kassanis, 1939). The number of consecutive infections given is rather small, but the results indicate that in this respect also the properties of the three viruses are similar. The experiments were carried out at the worst time of the year and this probably accounts for the low total percentage infection obtained, for according to Kassanis this virus is normally very readily transmitted by *M. persicae* in optimum conditions of preliminary fasting and short infection feeding.

Comparison between the infectivity of M. persicae *after discontinuous short feedings on a succession of healthy plants, and after feeding continuously on a single healthy plant for corresponding periods*

In the consecutive infection results already published for Hy 3 virus (Watson, 1936) it was found that when an infective aphid was fed on two successive healthy plants the number of second plants infected decreased with increasing time on the first healthy plant. The number of infections obtained by optimally infective aphids on the second plants were as follows:

	Feeding time on the first healthy plants			
	2 min.	5 min.	15 min.	1 hr.
No. of 1st plants infected out of 30	17	20	13	18
No. of 2nd plants infected	11	8	4	0

These results indicate that the insects were incapable of retaining their infectivity for as long as 1 hr. when feeding on a healthy plant, which agrees with the results of previous workers on similar lines. A low percentage of insects from some of the other treatments given in the same experiment did succeed in infecting a second plant after 1 hr. on the first, but the average decrease in infectivity was much more rapid than has been found for the discontinuous feedings described in this paper. It was therefore of interest to determine if the difference thus suggested was due to different experimental conditions, or whether the discontinuously fed aphides really retained their infectivity longer than those fed continuously for corresponding times. The procedure for this experiment was to feed a set of four aphides together for 2 min. on an infected leaf. They were then placed on four healthy plants, one of which was the first of a series of nine plants, and the others, (*a*), (*b*), and (*c*) were the first plants of three sets of two. When an aphid which was being given short consecutive feedings on the long series of plants was found to be feeding on the third of that series the corresponding aphid in set (*a*) was moved from the first plant to the second. When the discontinuously fed aphid had reached the sixth plant of its series the aphid was removed from the first to the second plant of set (*b*), and the aphid on the first

plant of set (*c*) was transferred to its second plant after the last transfer of the discontinuously fed aphid. The total results are given in Table 4.

TABLE 4. *Comparison between the infectivity of aphides during the course of a succession of short feeding periods on healthy plants, and that after corresponding time spent continuously on the first of two healthy plants. Total results for twenty-four repetitions of each treatment*

	Consecutive healthy plants								
	A	B	C	D	E	F	G	H	I
Discontinuous feeding	13	9	5	3	2	2	0	1	2
Continuous feeding: (*a*)	15	.....	3						
Continuous feeding: (*b*)	17	.....				0			
Continuous feeding: (*c*)	16	.....							0

The number of consecutive infections obtained on the long series of plants was smaller than in the previous experiments, but when the aphides were continuously fed on the first healthy plants the reduction of infectivity was still more rapid. Also, some of the aphides in the discontinuous series gave infection on the last plants on which they were fed, while the continuously fed insects failed to infect any of the second plants in the (*b*) and (*c*) series.

The rate of decline for the continuously fed aphides was more rapid than that exhibited in the 1936 consecutive infection experiments which were carried out in the autumn. For the discontinuous feedings, infectivities decreased more rapidly than in the experiments given in Table 1. This indicates that the observed summer reduction in the efficiency of the aphides may be due to more rapid inactivation of the virus, for, on analogy with the behaviour of such viruses *in vitro*, increases of temperature would tend to enhance the effect of an inactivating substance.

The discontinuously fed aphides penetrated the plant tissues nine times, and the continuously fed aphides, so far as could be determined from periodic observations of their behaviour, only once. Certainly it was rare for them to move from the leaf on which they were placed, and there was no evidence that they moved at all once they had settled down. Thus, loss of infectivity could not have been due to cleansing of the stylets from virus contamination, because, as the discontinuous feeding periods were all of the same duration, the same region of the stylets must have come into contact with the plant tissues on each occasion and the cleansing effect must have been far more rigorous than with the continuously fed insects. The fact also that several plants in the consecutive series, as in the previous experiments, were not infected, although the aphides fed on them, points to the conclusion that virus was present in the aphides, but was not, on these occasions, in the right position for injection into the plants. This is much the same explanation as Freitag put forward to explain similar results from the vectors of Curly-top virus. The retention of infective ability by the discontinuously fed aphides and not by the continuously fed ones supports the hypothesis put forward to explain the optimum infectivity of aphides after only 2 min. feeding on the infected plants, namely that 2 min. feeding does not stimulate the production of sufficient inactivating substance to affect the virus.

SUMMARY AND CONCLUSIONS

Individuals of *M. persicae*, when given an adequate fasting period followed by 2 min. feeding on the infected plants, can transmit *Hyoscyamus* 3, Potato *Y*, and Severe Etch viruses from tobacco to a number of successive healthy plants. The number of plants infected

seems to be that on which the insects can be given 2 min. feeding periods, within the time for which they would normally retain their infectivity if prevented from feeding at all. This is considerably longer than the time for which they would remain infective when feeding on a healthy plant. Rapid loss of infectivity of the vectors, especially when feeding, is a characteristic property of the type of virus used in these experiments which we have defined as "non-persistent" virus (Watson & Roberts, 1939). The present results confirm our previous hypothesis that loss of infectivity is not due to cleansing of the stylets when feeding, or to normal deterioration of the virus in the insects when fasting. The viruses appear to be inactivated by some substance produced by the aphides after they have been feeding for about 2 min. Persistent viruses, which are retained by their vectors for indefinite periods, are not affected by this substance, the vectors being able to acquire lasting infectivity by prolonged feeding upon infected plants, even when the virus available in the leaves is highly localized or very limited in amount. Most of the major differences between the results of transmission experiments with the two types of viruses could be accounted for by differences in the effect of the inactivating substance produced by the vectors. It is therefore unnecessary to postulate fundamentally different mechanisms of transmission between the two types.

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THE STRAWBERRY APHIS—*PENTATRICHOPUS* (*CAPITOPHORUS*) *FRAGARIAE* THEOB., WITH NOTES ON *P. POTENTILLAE* WALK. AND *P. TETRARHODUS* WALK.

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(With 5 Text-figures)

FOLLOWING the investigations of Davies (1932, 1934) and of Whitehead *et al.* (1932) on the biology of potato aphides in North Wales, it was decided to investigate the biology of the strawberry aphis, *Pentatrichopus* (*Capitophorus*) *fragariae* Theob., because of its importance as a vector of virus diseases of strawberries. Davies (1934) showed that where potato viruses spread only very slowly in areas such as the Lleyn Peninsula and Anglesey, their most important vector, *Myzus persicae* Sulz., occurred only in small numbers and often appeared late in the season. Davies (1937) also stated that there were in North Wales districts (such as Glanconway) where strawberries appeared to flourish. Particular reference has therefore been made to the possibility of finding areas in North Wales where strawberry runners will remain free from infestation by *Pentatrichopus fragariae*. Search for such districts has been made chiefly in the western parts of the province, where isolation from other strawberries is more easily obtained. The eastern part of the province includes the strawberry-growing district of Holt, in the Dee Valley, where strawberries appear to have degenerated on a scale comparable to that of other strawberry-growing areas. The observations have been made over a period of 2 years and have been extended to include the allied species *P. potentillae* Walk. and *P. tetrarhodus* Walk. These allied species were studied partly because of the confusion that exists in the synonymy and identification of species of the genus *Pentatrichopus*, and partly because of the possibility of their being able to live on strawberries. It has also been shown by Massee *et al.* (1937) and Hodson (1937) that *P. fragariae* will exist on such weeds as *Potentilla anserina* L. and *Fragaria vesca* L. The need for accurate identification becomes increasingly important because *Potentilla anserina* is the host plant of *P. potentillae*.

SYNONYMY

The synonymy of the strawberry aphis under the name *Capitophorus fragariae* has been discussed by Hodson (1937) who, however, does not deal with Ris Lambers's statement (1933) that the species should come in the genus *Pentatrichopus* of Börner. The genus *Pentatrichopus* is well defined—the first tarsal joints of adults have five hairs, the cornicles are comparatively long and thin and the antennae of the apterae are devoid of secondary sensoria (rhinaria). (The genus *Capitophorus* v.d. Goot has three hairs on the first tarsal joints, and the genus *Cryptomyzus* Oest. has apterae with small secondary sensoria on the third antennal segments.) Börner & Schilder (1932) consider *Pentatrichopus fragariae* Theob. to be a synonym of *P. fragaefolii* Ckll., and Ris Lambers (1933) states that both are synonyms of *P. pontentillae* Walk., but Ris Lambers now considers *P. potentillae* Walk. and *P. fragaefolii* Ckll. to be distinct species. The authors still think it possible that *P. fragariae* Theob. is a

synonym of *P. fragaefolii* Ckll., but more evidence on this point is required. It will be shown that *P. potentillae* Walk. and *P. fragariae* Theob. are certainly quite distinct species and easily separated. Specimens received from America and Canada taken on *Potentilla* spp. and *Rosa* spp. appear to differ from the above three species and have possibly been mistaken for, or described as, either *P. fragaefolii* Ckll. or *P. potentillae* Walk.

Bionomics of *Pentatrichopus fragariae* Theob.

(*a*) *Survey work*

The biology of this aphis in North Wales follows very closely that described by workers at East Malling (Massee *et al.* 1937) and Reading (Hodson, 1937). The aphis overwinters on strawberries in the apterous viviparous stage, but viviparous alatae and nymphs with wing buds have been found in late autumn and winter. Only one oviparous female has been found out of doors, but more are produced under glasshouse conditions. A few eggs have been found on strawberries, but it is not certain that they were the eggs of *P. fragariae*. In 1938, the summer generation of alatae appeared about the middle of May (seven were found on 100 leaves on 20 May), and migration continued until the beginning of July.

Detailed counts of the population of *P. fragariae* on strawberries have been made at Holt and Glanconway throughout the year. Counts have also been made at other isolated areas where small plots of strawberries are grown. The method of taking counts is similar to that used by Davies (1932) for potato aphides. From the whole of a plot, one fresh newly opened leaf was taken from 100 different plants. The aphides on these leaves were counted, and the number per 100 leaves is regarded as an index figure for the population of the plot. The accuracy of this method has been tested by Greenslade at East Malling; he came to the conclusion that the most satisfactory figure was obtained by choosing a partly opened young leaf for making these counts. The authors having started counts with newly opened leaves have continued to use this method in order to get comparative results.

Centre 1. *Holt, Denbighshire.*

Strawberries have been grown in the Dee Valley and especially at Holt since about 1860. The acreage of strawberries gradually increased until about 1910 or 1911 when some 700–750 acres of strawberries were cultivated. Since 1911 there has been a gradual deterioration of the crop in this area and the total acreage has now been reduced to 250 or less. Due to this degeneration, the acreage of one firm of growers has fallen from 360 to about 100. In February 1937, at the request of strawberry growers, a conference of growers and scientists was held at Holt and the possible causes of this degeneration were discussed. Numerous remedial measures have been adopted at this centre and attempts have continually been made to introduce vigorous healthy stocks, but with little success. Crop weights have continually decreased, and the variety Royal Sovereign especially has degenerated badly.

Field (*a*). Three-year-old Royal Sovereigns. By the summer of 1938 the strawberries in this field had degenerated very badly and they were ploughed up immediately after the 1938 crop had been picked. It will be seen (Fig. 1) that the population of *P. fragariae* increased rapidly between 12 April and 26 May when alatae were present.

Field (*b*). Three-year-old Royal Sovereigns. When first examined in 1938, the strawberries in this field had degenerated very badly in patches; they were ploughed up in early August. The aphis population increased only very slightly between 28 March and 12 April, but when next examined on 26 May a very sudden increase had taken place and this continued until 24 June. By 5 July the population had fallen from 1836 to 300 per 100 leaves. An attempt was made to burn the foliage after picking (between 5 July and 26 July); this did not affect the aphis count to any appreciable extent because large patches remained quite green and the index figure on 26 July was 144. Alatae were present on this field from 26 May until 5 July.

Field (*c*). Two-year-old Huxley Giant and Tardive de Leopold. This field was planted with alternate ten-row plots of the above varieties. It was examined for the first time on 12 April; no count was made but the aphis population was very high, particularly on Huxley Giant, even at this date. Both varieties looked healthy; the Huxleys in particular appeared to be growing very vigorously and were producing a large canopy of foliage, sheltering the young leaves on which large numbers of *P. fragariae* were breeding. A count was made for the first time on 26 May, when the very high index figure of 9748 was obtained on the Huxley Giant. After this date alatae were being produced in large numbers and by 24 June the index figure had dropped to 2940. Alatae were still present on 5 July, but no count was made. An extraordinary reduction in numbers had taken place by 26 July, when the index figure was only 24. It will be seen that after that date the aphis population on these plants never recovered; even during the summer of 1939 the highest figure recorded was 14 on 31 August. Counts on the Tardives followed a very similar trend, but the maximum index figure was only 678; this was recorded on 24 June, and by 26 July the population had fallen to 18 per 100 leaves. Subsequently the numbers remained very low, but late in the summer of 1939 there was a small peak population which rose above that on the Huxleys.

Field (*d*). Royal Sovereign and Tardive de Leopold. This field was planted with warm-water treated plants in the late autumn of 1937. These were thoroughly examined on 28 March and again on 12 April, when no *P. fragariae* were found. By 26 May, alatae had arrived on these plants and were breeding; on this date a count of 107 was obtained of which twelve were alatae. By 24 June a large increase had taken place and the index figure was 1797, with nine alatae; the population was still high on 5 July but, of 1756 aphides on 100 leaves, only two alatae were recorded. The 26 July count showed that some reduction had taken place and no alatae were found. The figure fell to 83 on 8 September and rose again to 134 on 28 September. On 13 February 1939, the index figure was 102, but on 18 April a further reduction had taken place and the figure was only 9. Another increase was recorded in 1939 when during June counts of 338 and 358 were recorded. Throughout the summer of 1939 no alatae and no nymphs with wing buds were found.

Field (*e*). This field was planted up with two separate plots of strawberries called Flat 1 and Flat 2. They were planted in isolation in an attempt to raise a healthy stock and were between large blocks of oats and potatoes. The nearest one (flat 1) was at least one mile distant from other strawberries. The plants were dipped in a pyrethrum insecticide and were planted in the spring of 1938. Flat 1 included the varieties Royal Sovereign, Oberschlesien, Noble, Cambridge Early, and Leopoldshall; flat 2 was planted with Royal Sovereign and Tardive de Leopold. The index figures for flat 1 include leaves from all the varieties; only the Royal Sovereigns were counted in flat 2.

On 26 May every leaf of fifty plants in flat 1 was carefully examined for the presence of *P. fragariae* and a large number of leaves from other plants in flats 1 and 2 were also examined. One alate female which had just dropped a nymph and one isolated small nymph were found. Subsequently the index figures rose to 700 on 19 August and fell to 156 on 28 September. The strawberries in flat 1 were planted under very adverse conditions; they made poor growth and were ploughed up in the autumn.

The index figures for flat 2 rose steadily to 664 on 28 September but by 13 February 1939 the population had fallen to 32 per 100 leaves. Both in March and April the index figures were 16; the highest figure for 1939 was 297 on 6 June. Throughout 1939, no alatae and only three nymphs with wing buds were found, two on 6 June and one on 16 June.

The index figures obtained for fields (*a*), (*b*) and (*c*) are plotted in Fig. 1. In the early summer of 1938 the population rose rapidly and in all three fields, alatae were being produced by 26 May. The period over which alatae were present is represented by the stippling. It is not known exactly when the first alatae were produced or when the last died out but the dates are probably a few days before 26 May and after 5 July respectively (i.e. the first and last records). In late June and July a very rapid decrease in the population was recorded; this was to a small extent accounted for by migration of alatae, but it was chiefly due to the action of predators and parasites including a parasitic fungus (probably *Empusa* sp.). On 26 May there were 1·2 % parasitized aphides on the Huxleys, this number had increased to 12·7 % by 24 June. Predators, particularly hover-fly larvae, considerably reduced the numbers but the greatest reduction was probably due to the fungus attack.

The 1939 population of *P. fragariae* in North Wales was very low and no alatae were found out of doors throughout the summer. This is in direct contrast to aphis populations generally in this year. The largest catch of aphides ever recorded in the mechanical trap at Aber, Bangor was taken in June 1939 (the trap has been working for five successive summers); it included a large number of different species and some colonized their host plants over a wide area. Fig. 1 shows that a slight increase in the population of *P. fragariae* took place in late summer but there was no definite high peak as in 1938.

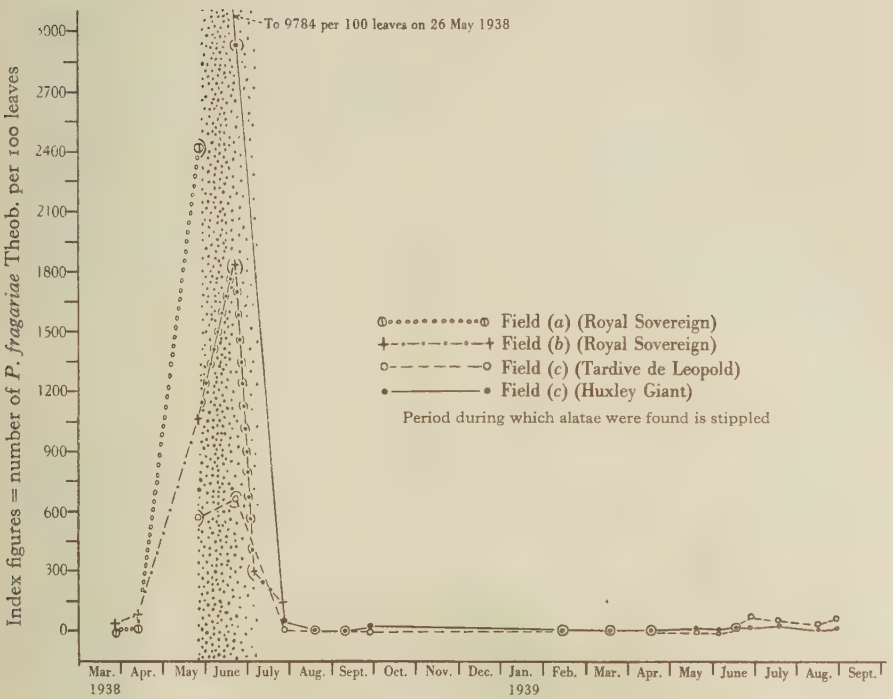

Fig. 1. Comparative populations of *P. fragariae* Theob. on strawberries in fields (*a*), (*b*) and (*c*), centre 1 (Holt, Denbighshire) in 1938 and 1939.

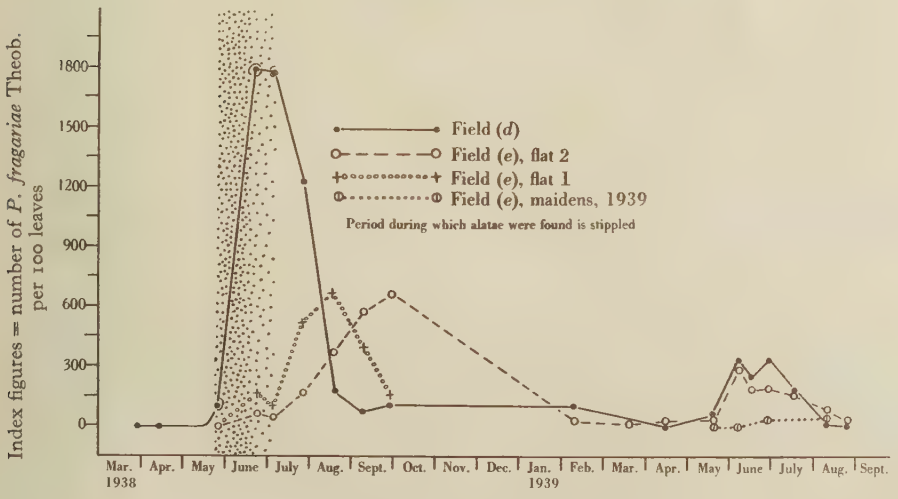

Fig. 2. Comparative populations of *P. fragariae* Theob. on strawberries in fields (*d*) and (*e*), centre 1 (Holt, Denbighshire) in 1938 and 1939.

Fig. 2 shows the counts in fields (*d*) and (*e*). Field (*d*), planted with treated runners in 1937, was adjacent to (*a*) and the highest population was recorded about one month later than on the older strawberries (see Fig. 1). From the beginning of July the numbers decreased rapidly and remained low until the summer of 1939 when a much smaller peak population was recorded. In the case of the maiden strawberries in field (*e*) flats 1 and 2, the peak population did not occur until mid-August and the end of September respectively; because of its isolated position the initial infection by alatae was much lower in this field. On 26 May only one was found on fifty plants (plus a large number of leaves from other plants), compared with twelve on 100 leaves in field (*d*). This is probably the reason for the later peak population. Flat 2 showing a peak population at the end of September was even farther from the source of infection. The successively later peak populations which appear to be correlated with the distance from a source of infection may to some extent be due to the difference in initial infestation as suggested above, but it may also be due to a delayed effect of predators and parasites in the more remote plots. In field (*d*) because of its nearness to the source of infection and also to the source of predators and parasites their effect would probably be felt sooner and the peak population would therefore occur earlier.

Centre 2. Glanconway.

Strawberries have been grown in the Conway Valley for a number of years. The present account applies to a farm which has a tradition for having grown flourishing crops of strawberries over a long period. The farm is on the east side of the River Conway and the field (field (*a*)) on which strawberries have most frequently been grown is on a south slope. Within the last 2 years the strawberries appeared to be degenerating rapidly and an effort has been made to grow healthy stocks obtained from Long Malling and Reading. These are being grown on a different part of the farm well isolated from other strawberries (field (*b*)).

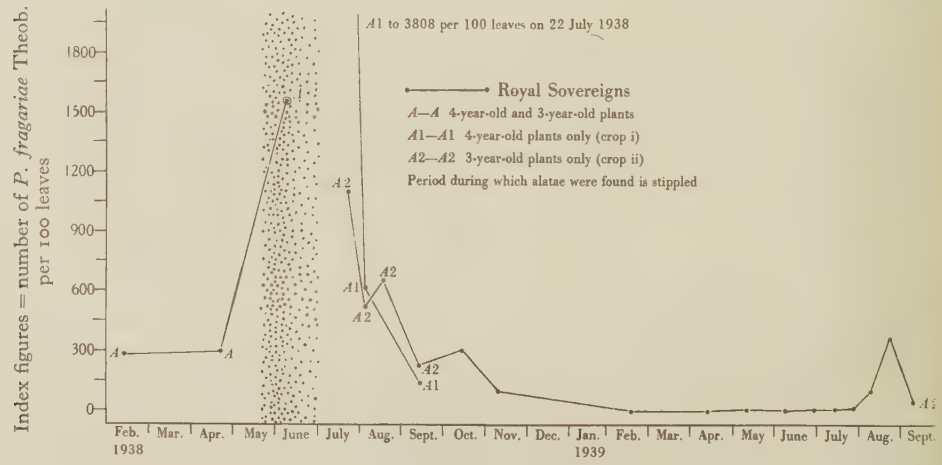
Index figures = number of P. fragariae Theob. per 100 leaves
1800
1500
1200
900
600
300
0
A1 to 3808 per 100 leaves on 22 July 1938
Royal Sovereigns
A—A 4-year-old and 3-year-old plants
A1—A1 4-year-old plants only (crop i)
A2—A2 3-year-old plants only (crop ii)
Period during which alatae were found is stippled
A
A2
A1
Feb. Mar. Apr. May June July Aug. Sept. Oct. Nov. Dec. Jan. Feb. Mar. Apr. May June July Aug. Sept.
1938
1939

Fig. 3. Comparative populations of *P. fragariae* Theob. on strawberries in field (*a*), centre 2 (Glanconway) in 1938 and 1939.

Field (*a*). In 1938 this field was being cropped with, (i) four-year-old Royal Sovereigns, (ii) three-year-old Royal Sovereigns, (iii) Oberschlesien. The index figures for these three crops are plotted in Fig. 3. The counts on crops (i) and (ii) were taken together until 22 July when a considerable difference in the aphis population on the two crops was noticed. Subsequently, counts were taken separately, and the dimunition in numbers was particularly rapid in crop (i) which was ploughed up in October. In crop (ii) there were 306 aphides per 100 leaves even on 14 October, which may probably be accounted for by the fact that the plants were making good growth and aphides increased in numbers even in September and the beginning of October. By November, however, the index figure had fallen to 95 and on 11 February and 11 April 1939 no aphides were found on 100 leaves. The subsequent breeding in 1939 took place very slowly and did not reach its peak until 22 August; even then the numbers were

far below the peak for 1938 (3808 on 22 June). On 8 June 1938, 44 alatae per 100 leaves were recorded; none was found in 1939. The population changes on the Oberschlesien (crop iii) were very similar to those on the Royal Sovereigns; the highest population however was not recorded until 5 August in 1938 and all figures for the year were below those for crops (i) and (ii).

Field (*b*). In 1938 this field consisted of one row of three-year-old plants originally obtained from East Malling and Reading. On one side of these were about fifty rows of two-year-old plants (which were taken as runners from the original row) and on the other side were about 2 acres of maiden plants obtained from the two-year-old plants. In 1939 a further fifteen rows of maidens were planted. The field is about a mile from field (*a*) (the nearest strawberries) from which it is separated by a hill about 200 ft. high.

In 1938 an attempt was made to trace the spread of aphides from the three-year-old plants to the maiden plants alongside. The maidens, when planted in early April, were almost free from *P. fragariae*. On 28 April fifty successive plants from each of four rows of the maidens were examined. The plants were in row 1 (adjacent to the three-year-old plants), row 7, row 15 and row 30. Each infested plant was noted and the examination repeated on 20 May, 22 July and 5 August. The results are recorded in Table 1.

TABLE 1. *The percentage of plants infested with* P. fragariae *at different dates*

	Percentage of plants infested			
Date	Row 1	Row 7	Row 15	Row 30
28 Apr.	22	33	5	0
20 May	76	—	35	7
22 July	76	94	100	60
5 Aug.	100	100	100	94

Before 28 April the infestation and spread was almost certainly due to the migration of apterae. On 20 May alatae were being produced but very few had migrated by this date so that it is probable that the increase up to 20 May was due to apterae. Between 20 May and 22 July alatae were partly responsible for increased infestation but the spread between 22 July and 5 August was again due to the movement of apterae.

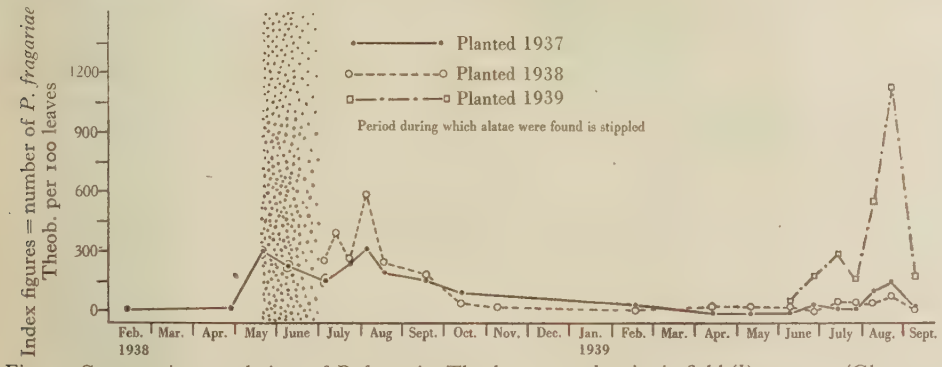
Index figures = number of P. fragariae Theob. per 100 leaves
1200
900
600
300
0
Planted 1937
Planted 1938
Planted 1939
Period during which alatae were found is stippled
Feb. Mar. Apr. May June July Aug Sept. Oct. Nov. Dec. Jan. Feb. Mar. Apr. May June July Aug. Sept.
1938
1939

Fig. 4. Comparative populations of *P. fragariae* Theob. on strawberries in field (*b*), centre 2 (Glanconway) in 1938 and 1939.

The index figures for field (*b*) are plotted in Fig. 4. In 1938 the peak population occurred in the two-year-old plants towards the end of June, but it did not occur in the maidens until 5 August. On both plots, the population then declined rapidly and even on 27 June 1939, it had risen only to 16 per 100 leaves. In this year, it reached its maximum towards the end of August but this was much lower than the 1938 peak. On the other hand, the population on the 1939 maidens increased rapidly from an index figure of 30 on 8 June to 1112 on 22 August. As in the instance already noted, the peak for the maidens was later than that for the older plants. Alatae were produced in fairly large numbers in 1938. On 20 May there were 7 per 100 leaves on the two-year-old plants but this had declined to 2 per 100 leaves on 8 June. It is not known how long the period of migration lasted at this centre because further examinations were not made until 4 and 13 July.

(*b*) *Establishment of experimental plots*

A small plot was first established near the laboratory in the autumn of 1937. This was planted with a number of varieties most of which were badly infested with *P. fragariae*. Half of these plants were warm-water treated before planting, but the aphis migrated quickly on to them and all were infested by the spring of 1938; this spread was all due to the movement of apterae. On 2 August 1938 two aphis-free Royal Sovereign plants were planted in this plot, one with its runners at least 9 in. and the other with its runners at least 3 ft. from the nearest infested strawberries. A third aphis-free plant was planted 12 yd. away from the plot. Plant 1 had been colonized on 15 August; at this date plants 2 and 3 were free. Plant 2 had been colonized by 23 August; plant 3 remained free. This is further evidence of the spread of apterae over bare soil for a distance of at least 1 yd.

A second plot was established near Madryn Farm Institute gardens on 11 April 1938. This was planted with one of the best commercial stocks of Royal Soverign runners obtainable, and all the plants were dipped in a nicotine solution. The plot remained free from *P. fragariae* throughout the summer, autumn and winter of 1938. Unfortunately, a stock of strawberries in the garden itself was infested and on 5 June 1939, two apterae of *P. fragariae* were found on the strawberries in this plot; it is not known how they were introduced but it is possible that they were carried there accidentally from the garden.

Soon after planting, symptoms of what appeared to be severe crinkle showed in the plants on this plot; they made a good recovery in the autumn but the symptoms again showed up in the summer of 1939. The plants were therefore destroyed in July.

A similar plot was established at Aberdaron in the corner of a grass field in an exposed situation facing the sea and isolated by several miles from the nearest aphis-infested strawberries. During the summer of 1938, an apparently healthy and vigorous stock of Royal Sovereigns was found in a garden near Chwilog (Lleyn); they had been obtained from a garden in Cardiganshire. Runners of this stock were planted in the Aberdaron plot but unfortunately most of them were destroyed by rabbits. The plants that remained and a few afterwards introduced continued free from *P. fragariae* throughout the summer of 1939. This plot has now been planted afresh with plants of the Chwilog stock. This stock also remained free from *P. fragariae* when planted in gardens at Bangor and Rhoscolyn, Anglesey. Rhoscolyn is in a windswept position on the coast near Holyhead and it is proposed to establish a larger field plot at this centre.

In the autumn of 1938 two plots of East Malling Royal Sovereigns were also planted about 200 yd. from the garden of Llysfasi Farm Institute, Denbighshire. These were dipped in nicotine solution before planting and one plot was sprayed with a nicotine wash 6 times at 4 day intervals beginning towards the end of May. Both plots have remained free from *P. fragariae*.

Host plants of *P. fragariae* Theob. *P. potentillae* Walk. and *P. tetrarhodus* Walk.

Since it has been shown by Massee *et al.* (1937) and Hodson (1937) that *P. fragariae* Theob. will live on *Potentilla anserina* L. (Silverweed), the possibility of this and other weeds acting as alternate host plants in the North Wales area has been investigated. Both *Potentilla anserina* and *Fragaria vesca* are very common members of the North Wales flora and another species of *Pentatrichopus*—*P. potentillae* Walk.—was found on Silverweed for the first time

in June 1938. In order to have more complete information on the host plants of the genus *Pentatrichopus*, the third British member, *P. tetrarhodus* Walk. which normally lives on roses was also included in the 1939 experiment. The results of experimental inoculations of various host plants in 1938 are recorded in Table 2 and the results in 1939 in Table 3. These results indicate that there is a clear-cut biological difference between the three species *P. fragariae* Theob., *P. potentillae* Walk. and *P. tetrarhodus* Walk. In all, strawberries were inoculated 12 times with *P. potentillae*; in no case did the species survive. *P. potentillae* also failed to live on *Fragaria vesca* L., *Fragaria moschata* Duch., *Potentilla sterilis* Garcke, *Potentilla reptans* L. and *Rosa rugosa* Thunb. *P. fragariae*, on the other hand, lived for varying lengths of time on all these host plants except *Potentilla reptans* and *Rosa rugosa*.[1] It also lived readily on *Potentilla anserina* and bred particularly well on *Fragaria vesca* and *Fragaria moschata*.

TABLE 2. *Results of inoculating various host plants with* Pentatrichopus fragariae *Theob., and* P. potentillae *Walk. in* 1938

	Aug.							Sept.						Oct.	
	2	12	15	18	23	26	29	1	3	7	11	17	20	11	15
P. fragariae:															
On strawberry	S	A	A	A+	A+	A+	A+	A+	N	A	N	N	A+	A	A
,, ,,	—	—	S	A	A	A+	A	A−	N	A	N	N	A+	N	N
On *Potentilla anserina*	S	A	A	A−	X	XR	A−	X	X	X	X	X	X	X	X
On *Potentilla sterilis*	S	XR	X	X	X	X	X	X	X	X	X	X	X	X	X
On *Fragaria vesca*	—	S	A	A	A	A	A+	A+	N	A+	N	N	A+	N	A
,, ,,	—	—	S	A	A	A	A	A+	N	A+	N	N	A	A+	N
On *Fragaria moschata*	—	—	S	A+	A+	A+	A+	A	N	A	N	N	A	N	N
P. potentillae:															
On *Potentilla anserina*	—	S	A	A	A	A	A+	A+	N	A+	N	N	A+	N	A
,, ,,	—	—	—	—	—	S	A	A	A+	A+	A+	A	A	AE	E
On strawberry	S	XR	X	X	X	X	X	X	X	X	X	X	X	X	X
,, ,,	—	S	X	X	X	R	N	X	X	X	X	X	X	X	X
,, ,,	—	S	A−	X	X	R	N	X	X	X	X	X	X	X	X
,, ,,	—	—	S	A	X	XR	X	X	X	X	X	X	X	X	X
,, ,,	—	—	S	A−	X	XR	X	X	X	X	X	X	X	X	X
,, ,,	—	—	S	A−	X	XR	X	X	X	X	X	X	X	X	X
On *Potentilla sterilis*	S	X	R	X	X	X	X	X	X	X	X	X	X	X	X
On *Fragaria vesca*	—	S	A	A−	X	R	X	X	X	X	X	X	X	X	X
,, ,,	—	—	S	A	X	XR	X	X	X	X	X	X	X	X	X
On *Fragaria moschata*	—	—	S	A	X	XR	X	X	X	X	X	X	X	X	X

S = initial inoculation. X = all dead.
A = alive. R = reinoculated.
A+ = breeding. N = no observation.
A− = weak, not breeding. E = eggs present.

P. tetrarhodus was included only in the 1939 experiment. It will be seen that this aphis lives very readily on strawberries and *Fragaria vesca*, not quite so well on *Potentilla sterilis*, and only for a very short time on *Potentilla anserina* and *Potentilla reptans*.

In North Wales, *P. fragariae* has only once been found in the field on *Potentilla anserina*—at Glanconway, on plants growing near infested strawberries; *P. fragariae* was found amongst

[1] In March 1940 alate *P. fragariae* colonized plants of *Rosa rugosa* under glasshouse conditions.

P. potentillae breeding on the same plant. Neither *P. potentillae* nor *P. tetrarhodus* has been found on strawberries; the latter has been noted only on its recorded host plants, i.e. species of the genus *Rosa*.

TABLE 3. *Results of inoculating various host plants with* Pentatrichopus fragariae *Theob.*, P. potentillae *Walk. and* P. tetrarhodus *Walk. in* 1939

	22 May	26 May	1 June	19 June
P. fragariae:				
On *Potentilla anserina*	S	A+	A+	A+
On strawberry	S	A	A	A
On *Potentilla reptans*	S	X	X	X
On *Potentilla sterilis*	S	A	A+	A−
On *Rosa rugosa*	S	X	X	X
On *Fragaria vesca*	S	A	A+	A+
On *Fragaria moschata*	S	A	A−	A+
P. potentillae:				
On *Potentilla anserina*	S	A	A+	A+
On strawberry	S	A−	X	X
On *Potentilla reptans*	S	X	X	X
On *Potentilla sterilis*	S	X	X	X
On *Rosa rugosa*	S	X	X	X
On *Fragaria vesca*	S	A−	X	X
On *Fragaria moschata*	S	A−	X	X
P. tetrarhodus:				
On *Potentilla anserina*	S	A−	X	X
On strawberry	S	A	A	A+
On *Potentilla reptans*	S	A−	A−	A−
On *Potentilla sterilis*	S	A−	X	X
On *Rosa rugosa*	S	A+	A+	D
On *Fragaria vesca*	S	A−	A+	A
On *Fragaria moschata*	S	A+	A	X

S = initial inoculation. A− = weak, not breeding.
A = alive. X = all dead.
A+ = breeding up. D = host plant dead.

It seems clear, therefore, that isolated plots of strawberries will not become colonized by *P. potentillae* and that *P. fragariae* is not likely to exist on *Potentilla anserina* except possibly in strawberry growing areas. *P. tetrarhodus* is an uncommon species and is not likely to be of economic importance.

IDENTIFICATION

During the course of the above experiments a few observations have been made on the life histories of *P. potentillae* and *P. tetrarhodus*. The former species produces alatae in early June and apterous oviparous females and apterous males in September and October. Eggs are laid in large numbers on the backs of the withering leaves, and these hatch in April. *P. tetrarhodus* has a similar life history and it is proposed to deal in more detail with the bionomics and morphology of the species in a subsequent paper. The three species can be separated by means of the following key:

A. Key to apterous viviparous females

1. (2) Antennae much shorter than body with segment III generally more than twice as long as IV. Cornicles generally shorter than antennal segment III. **P. tetrarhodus** Walk.
2. (1) Antennae nearly as long as body with segment III $1\frac{1}{4}$–$1\frac{1}{2}$ times as long as IV. Cornicles never shorter than antennal segment III.
3. (4) Not more than one knobbed hair on antennal segment IV; not more than five knobbed hairs on III, these very short, generally shorter than half diam. of III. **P. fragariae** Theob.

4. (3) More than one (generally four) knobbed hairs on antennal segment IV; more than five knobbed hairs on III, the longest nearly equal to diameter of III. **P. potentillae** Walk.

B. Key to alate viviparous females

1. (2) Sensoria on antennal segments III and IV. **P. fragariae** Theob.
2. (1) Sensoria on antennal segment III only.
3. (4) Antennae longer than or about equal to length of body with segment IV about two-thirds the length of III. Cornicles more than twice as long as cauda. **P. potentillae** Walk.
4. (3) Antennae considerably shorter than body with segment IV less than half the length of III. Cornicles only slightly longer than cauda. **P. tetrarhodus** Walk.

The above key brings out the main points of difference between the three species of *Pentatrichopus*, these and other important differentiating characters are tabulated below:

P. fragariae.

Apterous viviparous female. Antennae 1·2–1·5 mm. Not more than five knobbed hairs on IIIrd segment, these generally shorter than half the diameter of III. Not more than one very small knobbed hair on IVth segment. Hind tibia 0·6–0·74 mm.

Alate viviparous female. Antennae 1·55–1·85 mm. with a number of sensoria on IIIrd and IVth segments.

P. potentillae.

Apterous viviparous female. Antennae 1·7–1·9 mm. More than five knobbed hairs on IIIrd segment, longer than in *P. fragariae* and nearly equal to the width of III. IVth segment about two-thirds the length of IIIrd with more than one (generally four) knobbed hairs. Hind tibia 0·75–0·95 mm.

Alate viviparous female. Antennae 2·1–2·3 mm., longer or about equal to the length of the body; IVth segment, without sensoria, IIIrd with less than forty sensoria, about 1½ times as long as IV. Cornicles more than twice as long as cauda.

P. tetrarhodus.

Apterous viviparous female. Antennae 0·7–0·85 mm., more than eight knobbed hairs on IIIrd segment with at least one longer than the diameter of III: IVth segment generally less than half the length of third with more than one (generally four) knobbed hairs. Hind tibia 0·54–0·64 mm.

Alate viviparous female. Antennae 1·48–1·66 mm.: IVth segment without sensoria: IIIrd with more than forty sensoria and more than twice length of IVth. Cornicles very slightly longer than cauda.

The chief differences in the antennae and the comparative sizes of the hind tibia are shown in Fig. 5. It will be noticed that in *P. potentillae* the knobbed hairs on the proximal end of the hind tibia are longer and more numerous than in *P. fragariae*.

Other strawberry pests in North Wales

(*a*) *Other aphides.* During the course of the survey, aphides other than *P. fragariae* have been found on strawberries; some of these have bred freely on strawberries, others have occurred only as accidental migrants. In the latter category are the aphides, *Brachycaudus* (*Anuraphis*) *helichrysi* Kalt. and *Cavariella aegopodii* Scop. A few alatae and nymphs of *Macrosiphum rubiellum* Theob. have been found but the strawberry does not appear to be an important host plant of this species.[1] *Aulacorthum solani* Kalt. (*Myzus pseudosolani* Theob.) was frequently found on strawberries, particularly in the Holt district. This aphis breeds freely on strawberries but it was found chiefly at Holt where strawberries were growing near to potatoes. *Myzus ornatus* Laing (both alatae and apterae) has also been found on strawberries but only in very small numbers.

[1] *Macrosiphum rubiellum* Theob. overwintered as eggs on strawberries in North Wales in the season 1939–1940.

Other than *P. fragariae* the most abundant aphis has been *Macrosiphum solanifolii* Ashm. In 1938, this species was found in quite large numbers in spring and early summer and it bred until, in some districts, index figures of over 200 were recorded. In 1938 and 1939, the first specimens were found early in February, the greatest numbers occurred in April–early July and nearly all had migrated by mid July. It is possible that a few overwinter on strawberries, either as apterae or as eggs, otherwise it is difficult to explain their presence on the plants in early February.[1]

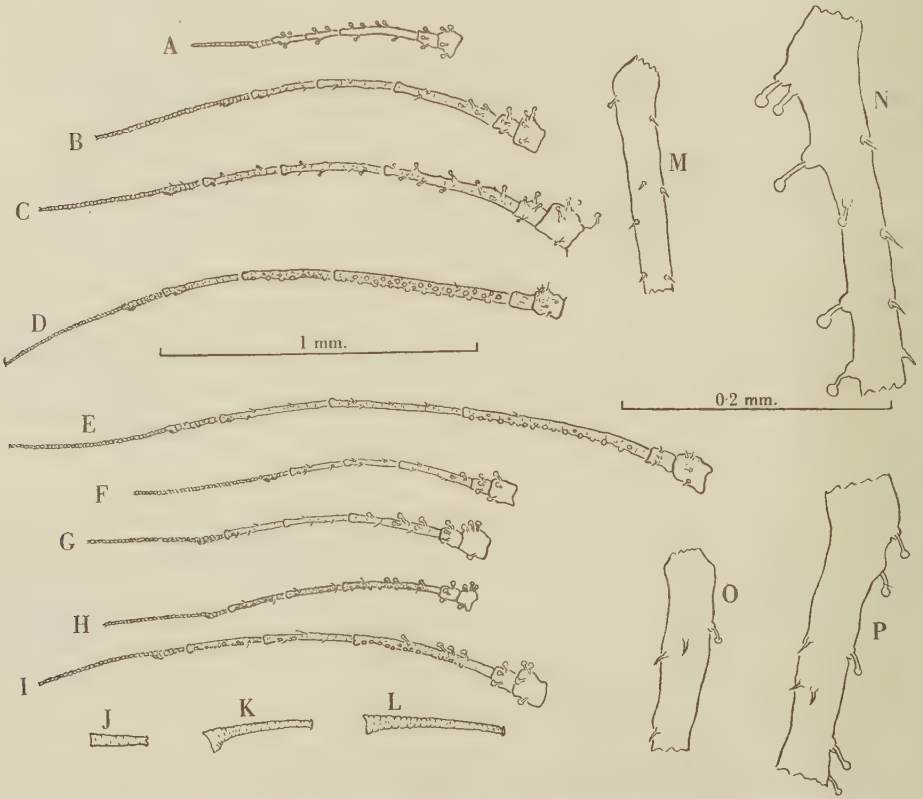

Fig. 5. A–I, antennae; A, *P. tetrarhodus* (apt. viv. ♀); B, *P. fragariae* (apt. viv. ♀); C, *P. potentillae* (apt. viv. ♀); D, *P. fragariae* (al. viv. ♀); E, *P. potentillae* (al. viv. ♀); F, *P. fragariae* (ovip. ♀); G, *P. potentillae* (ovip. ♀); H, *P. fragariae* (apt. ♂); I, *P. potentillae* (apt. ♂); J, K and L, cornicles of *P. tetrarhodus*, *P. fragariae* and *P. potentillae* (apt. viv. ♀♀) respectively. M and N, hind tibiae of apterous *P. fragariae* and *P. potentillae* respectively; O and P, hind tibiae of alate *P. fragariae* and *P. potentillae* respectively.

The index figures for aphides other than *P. fragariae* on strawberries are given in Table 4. The numbers recorded are very largely made up of *M. solanifolii*. Of the 236 aphides recorded on 19 May 1939 213 were *M. solanifolii* (1 alata, 8 nymphs with wing buds, 17 adult apterae and 187 immature apterae); 18 were *Aulacorthum solani* (5 adult apterae and 13 immature apterae) and 5 were other species.

[1] In the season 1939–40 *Macrosiphum solanifolii* Ashm. overwintered in the egg stage on strawberries at Holt and Bangor.

(*b*) *Tarsonemus pallidus* Banks. The strawberry mite has been prevalent at Holt and Glanconway and has also been found near St Asaph and at Madryn (Caernarvonshire). One crop of Royal Sovereigns suffered severe damage and the mite has occurred sporadically in other fields. Some of the older plants at Glanconway have been severely affected but the East Malling and Reading plants have remained free. The mite also occurs in the Lleyn Peninsula; in one garden in 1938, nearly every plant was attacked and badly stunted, the crop being practically worthless.

TABLE 4. *Index figures for other aphides* (*chiefly* Macrosiphum solanifolii *Ashm.*) *on strawberries*

	Centre 1		Centre 2			Centre 1		Centre 2	
	Field (*d*)	Field (*e*2)	Field (*a*)	Field (*b*)		Field (*d*)	Field (*e*2)	Field (*a*)	Field (*b*)
1938					1939				
11 Feb.	—	—	3	16	13 Feb.	0	5		
27 Apr.	—	—	300+		16 Feb.	—	—	0	10
20 May	—	—	—	160	17 Mar.	0	10	0	14
26 May	174				11 Apr.				
8 June	—	—	58	20	18 Apr.	50	64		
22, 23 June	122	78			10 May	—	—	11	0
4, 5 July	214	143	—	0	19 May	22	236		
22 July	—	—	0		6 June	34	18		
26 July	12	0			8 June	—	—	1	0
5 Aug.	—	—	0	0	16 June	16	3		
10 Aug.	—	—	0	0	27 June	—	—	1	0
19 Aug.	0	0			29 June	1	2		
8 Sept.	0	0			13 July	—	—	0	2
10 Sept.	—	—	0	0	18 July	0	4		
28 Sept.	0	0			26 July	—	—	1	0
14 Oct.	—	—	0	0	9 Aug.	—	—	2	0
9 Nov.	—	—	0	1	10 Aug.	0	3		
					23 Aug.	—	—	1	0
					31 Aug.	0	1		
					9 Sept.	—	—	0	0

(*c*) *Tetranychus telarius* L. Red spider has been recorded from most districts but has not been responsible for severe damage or reduction of crop, except in two fields at Holt. On one of these fields, it was extremely injurious and abundant in the summer of 1937 and the crop was much reduced. It was also severe in a field of maiden plants of Royal Sovereign and Pillnitz in the late summer and autumn of 1938.

No root weevils or other beetles have been found damaging strawberries during the course of the survey. Of the other important strawberry pests, *Acalla comariana* Zell., the strawberry *Tortrix* moth, has been found but is confined to a farm at Glanconway. *Lygus pratensis* F. has been noted on a few occasions but has never been numerous enough to cause anxiety.

DISCUSSION

The evidence so far obtained suggests that there are areas in North Wales where it will be possible to grow strawberries which will remain free from infestation by *P. fragariae* even in years when large numbers of alatae are produced. It must be borne in mind, however, that the plots which have so far remained free have been very small; increasing the size of the plots would increase the chances of infestation and further observations with larger plots are

needed. It has been shown that in strawberry growing areas such as Holt, aphis-free runners which are isolated by about a mile from the nearest strawberries become infested soon after migration starts in May. The introduction of aphis-free vigorous stocks into such areas is not likely to increase yields very considerably except in years such as 1939 when no alatae were produced. In 1938, alatae were present in strawberries for a period of over 6 weeks; if aphis-free plants could be thoroughly protected by spraying during this period, it is possible that the spread of virus diseases to a crop would be much reduced. Greenslade & Massee (1938) have recently shown that complete control of *P. fragariae* can be obtained using atomized nicotine. This, they state, is too dangerous and expensive for practical purposes, but if only one such spraying could be applied to plants immediately after migration had ceased, it would almost certainly result in a great reduction in the spread of virus diseases.

Summary

1. The genus *Pentatrichopus* Börner is well defined and the strawberry aphis should be referred to this genus.

2. The life history of *Pentatrichopus fragariae* Theob. in North Wales follows closely that described for other parts of the country. Alatae were numerous in May and June 1938 but none was found in 1939. The populations have been recorded as index figures (numbers per 100 young leaves) and these figures were much higher in 1938 than 1939.

3. Alatae migrate over a distance of at least 1 mile and there is considerable movement of apterae within a crop.

4. Small plots of strawberries isolated in non-strawberry growing areas remained free from *P. fragariae* in 1938 and 1939.

5. *P. fragariae* will live readily on *Potentilla anserina* L., *Potentilla sterilis* Garcke, *Fragaria vesca* L. and *Fragaria moschata* Duch. *P. potentillae* Walk. appears to live only on *Potentilla anserina*. *Pentatrichopus fragariae* has not been found on any weeds in non-strawberry growing districts.

6. A key is given for the separation of the species *P. fragariae* Theob., *P. potentillae* Walk., and *P. tetrarhodus* Walk.

7. Of other aphides found on strawberries in North Wales, *Macrosiphum solanifolii* Ashm. has been by far the most abundant. This species breeds very readily on strawberries in spring and early summer and it can overwinter on this crop.

8. Although *Pentatrichopus fragariae* is common on strawberries in most districts it does not appear to have a widespread migration in the same way as some other aphides, e.g. *Myzus persicae* Sulz. It is therefore thought that isolated plots in non-strawberry growing districts are likely to remain free from *Pentatrichopus fragariae* for long periods.

The authors are very much indebted to the Agricultural Research Council for a grant which covered the expenses involved in carrying out the investigation. They are also indebted to Mr R. J. V. Joyce, who helped with the work done up to July 1938; to Mr W. E. H. Hodson and certain American entomologists, for the loan of slides; to Dr H. Ris Lambers for aid with identifications and to Dr T. Whitehead and a number of growers and County Horticultural Instructors in North Wales for their interest and help.

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ON THE STEM AND BULB EELWORM (*ANGUILLULINA DIPSACI* KÜHN) WITH SPECIAL REFERENCE TO ITS OCCURRENCE ON WEEDS OF ARABLE LAND

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THE importance of wild hosts of *Anguillulina dipsaci* Kühn as carriers of infection needs no emphasis. The occurrence of the eelworm in the weeds, cleavers (*Galium aparine*) and chickweed (*Stellaria media*) taken in crops of oats and field beans which were themselves suffering from stem eelworm disease, was recorded by Johnson (1936) and Johnson & Thompson (1937); Walton (1937) confirmed the occurrence of infested cleavers plants in eelworm-infested oats. Walton also stated that experiments indicated that seed oats failed to carry eelworm infection, and from circumstantial evidence similar to that recorded by Johnson (1936) stated that "infestations of oat crops which occur, even after the absence of the crop for more than six years, are in all probability attributable to weeds rather than to infested seed". The present writer failed to obtain living eelworms from oat seed from infested crops in Yorkshire. This paper summarizes the results of field observations and experiments accumulated mainly during the past three years.

FIELD OBSERVATIONS

The following records are typical of many obtained during periodic visits to fields, and indicate the widespread occurrence of eelworm-infested weeds both in infested crops and in non-susceptible crops grown in the course of the rotation.

Field A. Cropping history: 1933 oats; 1934 barley; 1935 seeds; 1936 potatoes; 1937 oats. The 1933 oat crop suffered badly from stem eelworm disease; in 1937 infection occurred in the oats, and in cleavers and chickweed in the field.

Field B. In 1929 the field carried an oat crop badly attacked by stem eelworm. Oats were omitted from the rotation until 1939 when no signs of tulip-root symptoms could be seen in the oats but infested cleavers plants were obtained. Specimens of chickweed collected were free from infection.

Field C. Cropping history: 1936 barley; 1937 oats; 1938 wheat; 1939 roots. The oats were infested in 1937 and infested cleavers and chickweed plants were obtained in 1936, 1937, 1938 and in 1939.

Field D. Cropping history: 1936 roots, 1937 barley, 1938 oats, peas and tares. A few infested cleavers plants were collected in 1936. In 1938 there was some infection in the oats and specimens of infested cleavers and chickweed were easily found.

Field E. Cropping history: 1936 oats, 1937 seeds, 1938 sugar beet, 1939 wheat. The 1936 oat crop was infested by the eelworm and in 1939 specimens of infested cleavers were readily obtained.

In the case of field beans, fewer visits to known centres of infection were made, but the writer recorded (1936) the occurrence of widespread infection of cleavers seedlings growing in fallow soon after the ploughing in of a portion of a bean crop which had virtually failed as a result of stem eelworm disease. Fields visited in years subsequent to bad eelworm attacks yielded infested cleavers plants in the alternative rotational crops. In one field, for example, the cropping history was: 1936 beans, 1937 potatoes, 1938 wheat, 1939 barley. Each year infested cleavers plants were obtained.

Infection experiments

The following account includes the results of experiments designed to test the infectivity of various strains of the eelworm on different hosts. The plants were raised in eelworm-free soil in ordinary pots sunk in the ground, or in large glazed pots placed on the surface. Infected dried plant material was added to the soil prior to sowing. Adequate controls were included, treatments were replicated and each type of experiment was repeated at least once in subsequent seasons. The cultivated hosts beans and oats, in early experiments, were subjected to *A. dipsaci* infection derived from oats, beans, cleavers and chickweed. With the chickweed strain it was difficult to obtain a considerable amount of added infection by the addition of a reasonable amount of dried chickweed and in subsequent trials, therefore, this strain was omitted from the series. Positive infection was, however, obtained in beans and oats from chickweed and reciprocal infection, viz. oat strain and bean strain infection of chickweed, was also recorded. With the other three strains, viz. those derived from oats, beans and cleavers, heavy infection occurred in the cultivated plants both when the plants were grown separately in the experimental pots, and also when oats and beans were sown together in the same pot. Thus in experiments conducted in 1938 and repeated in 1939, 100% infection of oats and beans subjected to the oat and the bean strains respectively occurred. Both strains produced equally severe tulip-root symptoms in oats. With the cleavers strain 50% of the oat plants and 75% of the bean plants were infected. This smaller degree of infection was doubtless due to the smaller amount of infection added in the infective cleavers material, and on this account tulip-root symptoms were noticeably less severe. In beans certain symptoms of infection were very pronounced. Thus, infected plants were always markedly discoloured reddish to reddish black at the base of the plant, basal side shoots were abnormally numerous and, in the later stages of growth, browning of the pith frequently ensued.

Infection of cleavers plants by the bean, oat and cleavers strains was also obtained in similar experiments. The finding of infested cleavers seedlings in fallow after ploughing in an infested bean crop has already been mentioned. Similar conditions were reproduced experimentally by sowing cleavers seeds in early September in four pots which had been used to test the infectivity respectively of the oat, bean, cleavers and chickweed strains on a mixed crop of beans and oats. After harvesting the crop, five cleavers seeds were sown in each pot, and the seedlings removed in early November. In spite of the removal of all the infected cultivated plants infection occurred in the cleavers seedlings. Four seedlings were infected in the bean strain pot and three seedlings were infected in the case of the other three strains.

It seems clear from the above results that a relationship exists between the strains of *A. dipsaci* occurring in beans, oats, cleavers and chickweed in the localities visited in Yorkshire, and that there is no doubt that the weeds act as carriers of eelworm capable of infesting the cultivated crops. The weed cleavers in the writer's experience is in many districts very widespread on cultivated land, and fields have been visited after harvest when the surface of the land under stubble was literally covered by the seeds of this weed. With the possibility in the future of more intensive arable cropping, and in particular cereal cultivation, the potential danger from such weeds as carriers of *A. dipsaci* in localities where the eelworm is known to occur should be emphasized. It is clear that reasonable rest from a susceptible

crop without proper attention to clean cultivation may not be sufficient to prevent serious outbreaks of stem eelworm disease.

The above conclusions raise interesting questions and invite criticism concerning the subject of biologic strains of *A. dipsaci*, especially in view of the findings of Steiner (1925) and Hodson (1931). The researches of these workers show that it is unwise to label, for example, a strain of *A. dipsaci* from oats, as the "oat strain", since oat strains may be obtained which may vary in their powers of selection of hosts upon which they can feed. This power of host selection would depend upon the past history of parasitism of the eelworm. Under conditions prevailing in the field in the localities in Yorkshire concerned in the present paper, it seems safe to assume that the so-called strains encountered on field beans and oats are reciprocally infective, and that the weed strains mentioned are capable of parasitizing the cultivated hosts both when they have the choice of the two cultivated hosts and when opportunity of infecting one host only is provided. Conditions similar to those encountered in Yorkshire are likely to prevail in other parts of the country, and Walton's confirmation of the occurrence of infested cleavers specimens and certain other weeds among infested oats in Somersetshire bears out this suggestion.

Opportunity is taken to record here certain other observations. The regular occurrence of *A. dipsaci* in adventitious chickweed plants in experimental pots and cylinders in which various hosts were subjected to infection by a rhubarb strain of the eelworm have already been recorded (Johnson, 1939). The reciprocal infectivity of strains from oats and rhubarb were also recorded. In recent experiments, it has been found that the rhubarb strain which is apparently not rigidly restricted in its feeding habits is capable also of transference on to field beans, and some evidence is also available that a strain from field beans recorded as attacking oats will infect rhubarb.

These findings suggest that some relationship exists between these various so-called strains, on the three cultivated hosts, since they are reciprocally infective and can also pass on to similar wild hosts.

It should also be noted that infective rhubarb material is commonly obtained from rhubarb in which the eelworm has been breeding undisturbed for two to three years.

Summary

Field observations show the widespread occurrence of *A. dipsaci* on the weeds cleavers (*Galium aparine*) and chickweed (*Stellaria media*) in eelworm-infested oats and on cleavers in infested crops of field beans. The infestation in these weeds persists in subsequent years in the fields under non-susceptible rotational crops.

Infection experiments show that strains of *A. dipsaci* occurring on oats and field beans are reciprocally infective, and that the weed strains are capable of infesting the cultivated hosts and vice versa. The weed hosts act, therefore, as reservoirs of infection, maintaining eelworm infestation in fields in spite of rotational cropping.

Experiments prove that the rhubarb strain of *A. dipsaci* which readily infests oats and chickweed is capable also of transference on to field beans.

The writer wishes to thank his colleague, Mr H. W. Thompson, for his assistance in affording the opportunity of visiting the farms under observation.

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LABORATORY TESTS WITH LIQUID INSECTICIDES ON THE EGGS OF THE BED-BUG, *CIMEX LECTULARIUS* L.

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(With 10 Text-figures)

EXPERIMENTS on the ovicidal efficiency of liquid insecticides to the bed-bug and the possibilities of eggs of the bug as material for these experiments appear to have been neglected. For eradication of the bed-bug, Nijkamp & Swellengrebel (1934) used a pyrethrum liquid, Schedl (1935) found a liquid chlorinated hydrocarbon effective, and Kemper (1936) details various proprietary and other liquids. The bed-bug is usually in hiding in cracks during the application of liquid insecticides, which, in consequence, have been less popular than fumigants, but those liquids which are capable of exerting residual effects might be of practical value. Potter (1938) showed that insects were incapacitated by contact with certain sprayed surfaces and Potter & Musgrave (1939) found that an organic thiocyanate, β-butoxy-β′-thiocyanodiethylether (*n*-butyl-carbitol-rhodanate), was effective as a contact spray, had a fumigant effect, and could be made to form a toxic film; subsequently this thiocyanate has shown considerable promise in some tests in bug-ridden houses (Musgrave, 1940).

Thiocyanates have been tested against industrial and household insect pests other than the bed-bug. Badertscher (1936) compared pyrethrum, rotenone and β-butoxy-β′-thiocyanodiethylether as fly sprays; Woodbury (1938) found that rotenone and pyrethrum were superior to β-butoxy-β′-thiocyanodiethylether against cockroaches, and Tischler & Viehoever (1938) claimed that α-naphthyl-isothiocyanate had certain advantages over β-butoxy-β′-thiocyanodiethylether. Hitherto, lauryl-rhodanate, α-naphthyl-isothiocyanate, β-butoxy-β′-thiocyanodiethylether and pyrethrins have not been compared as insecticides against the same biological material. Lauryl and β-butoxy-β′-thiocyanodiethylether have apparently never been compared as insecticides for household use. Detailed laboratory tests, whereby these various insecticides were compared with a known degree of accuracy, are the subject of this paper.

MATERIALS

The compositions of the liquid insecticides used are given in the account of the results. The liquids were diluted to the concentrations required by mixing them with a highly refined kerosene-type oil of the following specification:

Specific gravity	0·778
Flash-point (closed)	158° F.
Initial boiling-point	200° C.
Final boiling-point	270° C.
Colour	Water white
Sulphur content	0·05 %
Odour	No odour of kerosene
Viscosity Redwood 1 at 60° F. ...	32″.

This oil is marketed as "Odourless distillate".

Technique

Bed-bugs, *Cimex lectularius* L., were bred in 2 × 1½ in. tubes of thick glass at a temperature of 23° C. and a relative humidity of approximately 70 %. Eggs were obtained by providing the tubes with blotting paper on which the insects oviposited. Usually eggs were used in experiments 2–5 days after they had been laid. The stage of development reached by the eggs within the female is variable (Johnson), so that their true age is uncertain.

TABLE 1. *Results of spraying experiments expressed as equations for the calculated regression lines. When $P < 0.05$ there is heterogeneity in the results*

n^1 = number of degrees of freedom = $n - 2$, where n is the number of separate determinations used to calculate the regression line.
h = heterogeneity, i.e. ***$P < 0.01$
*$P > 0.05$.
χ^2..., P..., Fisher, Table 2 (1936).

Insecticide	Regression equation	n^1	χ^2	P	Av. no. of eggs	h
β-butoxy-β′-thiocyanodiethylether 3 %	$Y = 5.56 + 4.93\ (X - 1.15)$	38	111.772	< 0.01	59.9	***
β-butoxy-β′-thiocyanodiethylether 2 %	$Y = 5.28 + 4.38\ (X - 1.40)$	12	9.842	> 0.6	54.8	*
α-Naphthyl-isothiocyanate 3 %	$Y = 5.44 + 4.09\ (X - 1.29)$	5	4.9725	> 0.3	55.0	*
Mixture { β-butoxy-β′-thiocyano-diethylether 1 % + Pyrethrins 0.1 %	$Y = 5.08 + 4.30\ (X - 1.37)$	6	8.976	> 0.1	80.0	*
Pyrethrins 0.4 %	$Y = 5.35 + 1.87\ (X - 1.35)$	18	38.805	< 0.01	70.4	***
Pyrethrins 0.1 %	$Y = 4.63 + 2.37\ (X - 1.47)$	9	4.704	> 0.8	49.6	*
Dodecyl-thiocyanate 3 %	$Y = 4.40 + 2.41\ (X - 1.52)$	6	3.525	> 0.7	45.7	*
"Odourless distillate"	$Y = 4.26 + 2.50\ (X - 1.63)$	17	56.118	< 0.01	54.0	***

Average control mortality = 5.036 % ± 2.851 %.

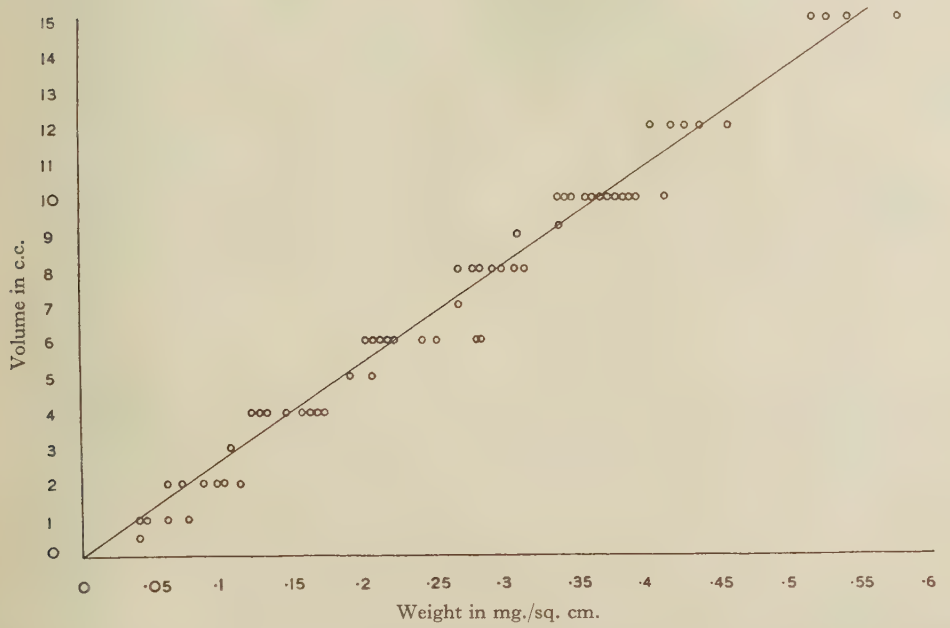

Fig. 1. The relationship between the volume of liquid placed in the reservoir of the spray gun and the weight deposited on the basal plate of the spraying tower. The dots are the plotted points. See Table 1.

Experiments suggested that there was no appreciable difference in the susceptibility of eggs at ages between 0 and 7 days. Adequate randomization of the eggs was impossible because they could not be removed undamaged from the blotting paper.

Eggs were sprayed in a specially constructed spraying tower (see Potter & Musgrave, 1939; Tattersfield, 1939, p. 366). The advantages of this apparatus are that it is simple to use and gives an almost even deposit on the surface of the basal plate which receives the spray. The atomizing gun of this apparatus was supplied with air at 25 lb./in.^{2} by means of a compressor and a reducing valve. Evidence suggested that with a constant speed of flow of the liquid through the gun, the weights deposited on a unit area were directly proportional to the volume of liquid put into the gun. This characteristic, which subsequently proved a little unreliable, was used in an attempt to administer accurately known dosages of insecticide: known volumes of "Odourless distillate" were atomized into the spraying tower and the weight deposited on four $\frac{7}{8}$ in. glass cover-slips placed on the basal plate was determined for each volume (Table 1) and a volume-weight graph was drawn. The graph was constructed from several series of determinations made at different times. The plotted points fell on a straight line passing through the origin; a provisional line was drawn (Fig. 1) and from it could be determined the weight deposited by any given volume of liquid in mg./sq. cm. Subsequently, the best line passing through the plotted points and the origin was calculated and found to differ only slightly from the provisional line.

In our experiments the concentration of insecticide has been kept constant and the dosages varied. This method is simpler and easier in operation than that in which the dosage is kept constant and the concentrations are varied, as tried in earlier work (Potter & Musgrave, 1939). Work of Shackell (1925) and Shepard & Richardson (1931) suggests that the two methods would give essentially similar results.

The bugs laid eggs on both sides of the blotting paper in the tubes. For spraying, eggs were removed from that side of each sheet on which fewer eggs had been laid, and those remaining were used in the experiments. For each dose of insecticide sufficient sheets to provide from 50 to 100 eggs were taken, placed on the basal plate of the tower and sprayed with a known volume of insecticide. The speed of flow of the liquid through the gun was determined with a stopwatch. The eggs were left in the tower for 1 min. after the total volume had been sprayed; they were then transferred to $2 \times 1\frac{1}{2}$ in. tubes with muslin covers and kept for at least 10 days at a temperature of 23° C. and a relative humidity of approximately 70 %. Several experiments were performed to determine the time required for eggs to hatch and on all occasions it was found that live eggs had all hatched 10 days after spraying.

By counting the eggs and the completely hatched nymphs it was easy to determine the percentage kills. Some nymphs died before completely emerging from the eggs; these were always counted as dead.

By means of the volume weight curve mentioned above, the dosages of insecticides were expressed as approximate amounts of liquid deposited in mg./sq. cm. The percentage kills were converted to "probit" units (Bliss, 1935*a*) which were plotted against logarithms of the dosages. For each insecticide (except in the supplementary experiments) an accurate regression line was calculated (Table 1) and the limits of error for odds of 19 to 1. The data, regression lines and limits of error are shown graphically in Figs. 2–9. Fig. 10 shows all the regression lines on one graph for comparison.

Legends for Figures 2–9.

Fig. 2. The regression line for 3 % β-butoxy-β'-thiocyanodiethylether with the limits of error shown by the dotted lines and the forty plotted points. Abscissa: probits. Ordinate: log dose, mg./sq. cm. + 2.

Fig. 3. The regression line for 2 % β-butoxy-β'-thiocyanodiethylether with the limits of error shown by the dotted lines. Abscissa: probits. Ordinate: log dose, mg./sq. cm. + 2.

Fig. 4. The regression line for 3 % α-naphthyl-isothiocyanate with the limits of error. Abscissa: probits. Ordinate: log dose, mg./sq. cm. + 2.

Fig. 5. The regression line for an insecticide consisting of 1 % β-butoxy-β'-thiocyanodiethylether and 0·1 % pyrethrin. Abscissa: probits. Ordinate: log dose, mg./sq. cm. + 2.

Fig. 6. The regression line for 0·4 % pyrethrin. Abscissa: probits. Ordinate: log dose, mg./sq. cm. + 2.

Fig. 7. The regression line for 0·1 % pyrethrin. Abscissa: probits. Ordinate: log dose, mg./sq. cm. + 2.

Fig. 8. The regression line for 3 % dodecyl thiocyanate. Abscissa: probits. Ordinate: log dose, mg./sq. cm. + 2.

Fig. 9. The regression line for "odourless distillate". Abscissa: probits. Ordinates: log dose, mg./sq. cm. + 2.

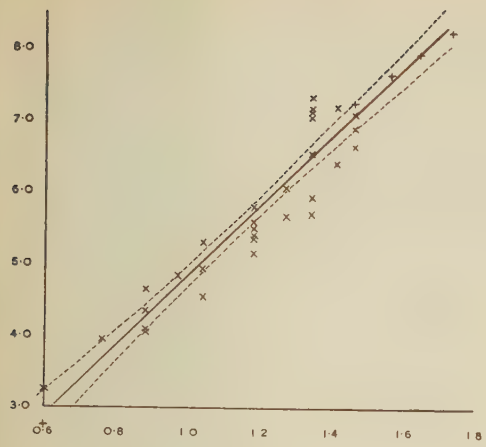

Fig. 2.

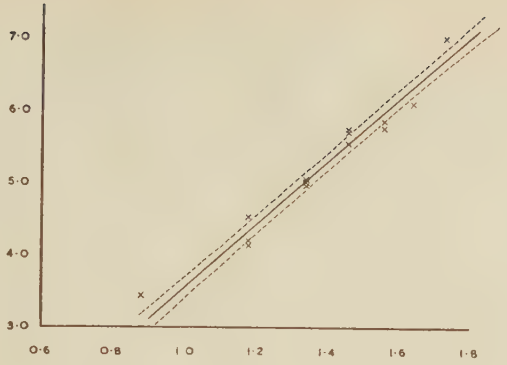

Fig. 3.

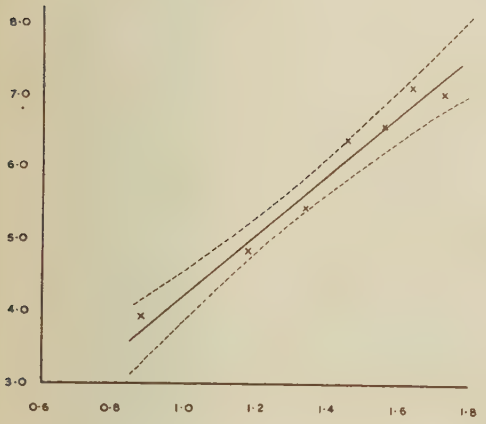

Fig. 4.

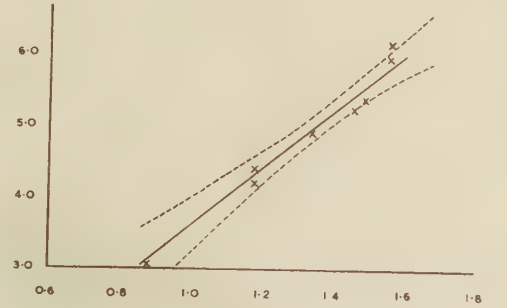

Fig. 5.

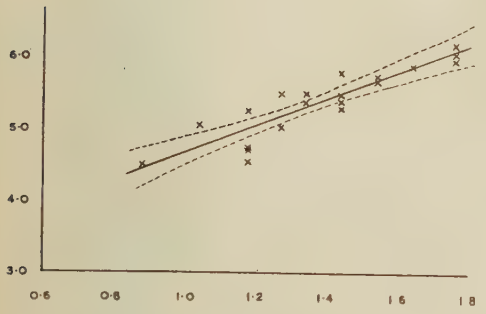

Fig. 6.

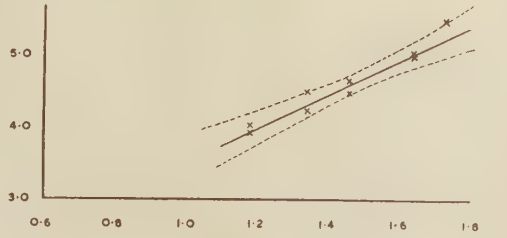

Fig. 7.

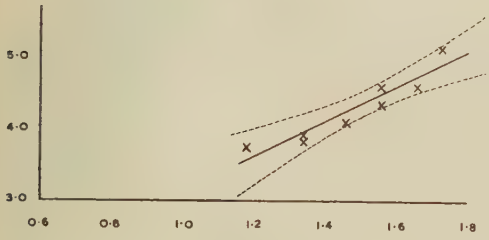

Fig. 8.

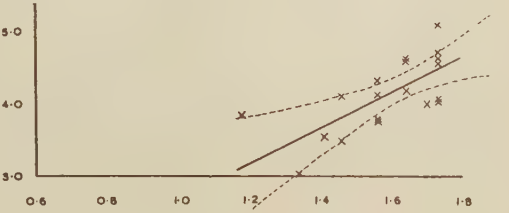

Fig. 9.

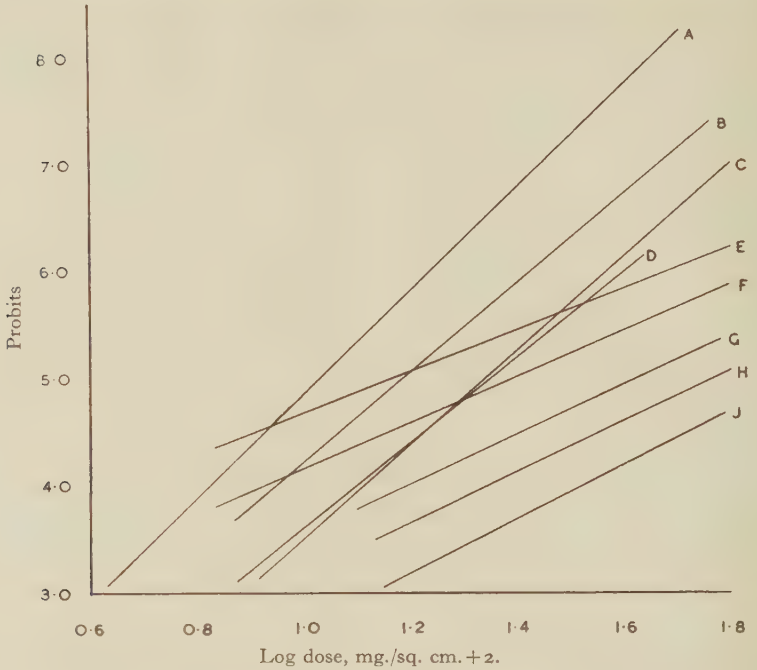

Fig. 10. The regression lines for the various liquids are drawn on one graph for comparison. For greater clarity the plotted points and the limits of error are not shown. A. 3 % β-butoxy-β'-thiocyanodiethylether. B. 3 % α-naphthyl-isothiocyanate. C. 2 % β-butoxy-β'-thiocyanodiethylether. D. Mixture of 1 % β-butoxy-β'-thiocyanodiethylether and 0·1 % pyrethrins. E. 0·4 % pyrethrins. F. Isobutyl-undecylenamide and pyrethrins. Mixture not discussed in this paper. G. 0·1 % pyrethrins. H. 3 % dodecyl-thiocyanate. J. "Odourless distillate."

Results

When making comparisons between various substances to be used as insecticides in commercial practice it is obvious that such matters as health hazard and cost of materials are of importance and we have taken these into account in making up the solutions used in our experiments.

A. β-butoxy-β'-thiocyanodiethylether is marketed as 50 % solution in a kerosene-type oil under the name of "Lethane 384" (Murphy, 1937). 3 and 2 % solutions of the thiocyanate were obtained by making 6 and 4 % solutions of Lethane 384 respectively.

To determine the regression line for 3 % β-butoxy-β'-thiocyanodiethylether an average number of 59·875 eggs was used in forty separate sprayings. The forty points are plotted in Fig. 2, and the regression line $Y = 5{\cdot}56 + 4{\cdot}93\ (X - 1{\cdot}15)$, with its limits of error, is shown. For 38 degrees of freedom $\chi^2 = 111{\cdot}772$, and P is well below 0·01 (Fisher, 1936).

An average number of 54·857 eggs was used in fourteen sprayings to determine the regression line for 2 % β-butoxy-β'-thiocyanodiethylether. The points are plotted in Fig. 3, and the regression line $Y = 5{\cdot}28 + 4{\cdot}38\ (X - 1{\cdot}40)$ with its limits of error is shown. χ^2 for 12 degrees of freedom = 9·8423 and $P > 0{\cdot}6$.

B. α-Naphthyl-isothiocyanate is marketed under the trade name "Kesscocide". It is a pure white crystalline compound and a 3 % solution in odourless distillate was made.

An average number of 55·0 eggs was used in experiments to determine the regression line. The points are plotted in Fig. 4 and the regression line $Y = 5{\cdot}44 + 4{\cdot}09\ (X - 1{\cdot}29)$, with its limits of error, is shown. χ^2 for 5 degrees of freedom = 4·9725 and P lies between 0·3 and 0·5.

C. An average number of eighty eggs was used in eight sprayings to determine the regression line of a mixture of 1 % β-butoxy-β'-thiocyanodiethylether and 0·1 % pyrethrin. The points are shown in Fig. 5, and the regression line $Y = 5{\cdot}08 + 4{\cdot}3\ (X - 1{\cdot}37)$, with its limits of error, is shown. χ^2 for 6 degrees of freedom = 8·9758 and P is between 0·1 and 0·2.

D. The pyrethrins were obtained from a proprietary oleoresin preparation marketed under the name of "Pyefly" which gave a 0·1 % solution of pyrethrins (approx.) at a dilution of 1–64; the 0·4 % solution was obtained by diluting 1 part of the oleoresin with 16 parts of "odourless distillate".

An average number of 70·45 eggs was used in twenty separate sprayings to determine the regression line for 0·4 % pyrethrins. The points are plotted in Fig. 6 and the regression line $Y = 5{\cdot}35 + 1{\cdot}87\ (X - 1{\cdot}35)$, with its limits of error, is shown. χ^2 for 18 degrees of freedom is 38·805 and P is less than 0·01.

The 0·1 % pyrethrins solution was obtained by diluting the pyrethrin preparation with 64 parts of "odourless distillate".

An average number of 49·635 eggs in eleven separate sprayings was used to determine the regression line. The points are plotted in Fig. 7 and the regression line $Y = 4{\cdot}63 + 2{\cdot}37\ (X - 1{\cdot}47)$, with its limits of error, is shown: χ^2 for 9 degrees of freedom is 4·7038 and P lies between 0·8 and 0·9.

E. Dodecyl-thiocyanate was used as a 3 % solution in odourless distillate.

An average of 45·75 eggs in eight sprayings was used to determine the regression line. The points are plotted in Fig. 8 and the regression line $Y = 4{\cdot}4 + 2{\cdot}41\ (X - 1{\cdot}52)$, with its limits of error, is shown. χ^2 for 6 degrees of freedom = 3·5253 and $P > 0{\cdot}7$.

F. Odourless distillate being used as the carrier, an evaluation of this material alone was usually included in each series of sprayings with the other insecticides. Also separate experiments were performed to determine its toxicity.

An average of 54·0 eggs was used in nineteen sprayings to determine the regression line. The points are plotted in Fig. 9 and the regression line $Y = 4{\cdot}26 + 2{\cdot}50\ (X - 1{\cdot}63)$, with its limits of error, is shown. χ^2 for 17 degrees of freedom is 56·118 and P is well below 0·01.

All the regression lines, without the points and limits of error, are shown for comparison in Fig. 10.

Bliss (1935*a*) considers that a value of χ^2 such that P is less than 0·05 indicates heterogeneity in the results attributable to other factors than errors of random sampling, but that the results may nevertheless be of use for comparison with other toxicological data.

Supplementary experiments

G. In one experiment 65 eggs were "sprayed" for 150 sec. under the usual conditions except that no liquid was put in the gun. The standard speed of flow was 1 c.c. in about 36 sec., so 180 sec. represented the time taken by 5 c.c. A kill of 9·2 % was obtained but this was not significantly greater than might have been obtained from the controls. (See average control mortality in Table 1.) The kill in the control for this experiment was 4·9 % (61 eggs).

H. An experiment was designed to discover the susceptibilities of eggs of different ages. Eggs aged 0–3 days and 2–5 days were sprayed on the same day with 0·4% pyrethrins in "odourless distillate" at three dosages. The results are shown in Table 3 and from these data it was determined that there was no significant difference in the susceptibilities of the eggs, a result which was supported by a similar experiment, with 3% β-butoxy-β′-thiocyanodiethylether, in which, however, the experimental points were rather scattered.

Relative "potencies" of insecticides

The regression lines for the eight insecticides were separated into two groups (Fig. 10), the lines in one group having steeper slopes than those in the other, and this difference in slopes was shown to be mathematically significant by a method of Bliss (1935*b*), which also showed that the lines in each group did not differ significantly in slope. A value of P for each pair of regression lines was read from Fisher's (1936) t table by entering the value of t_b, obtained from the expression

$$t_b = \frac{b_1 - b_2}{\sqrt{\{V(b_1) + (b_2)\}}},$$

with n equal to the sum of the degrees of freedom in the two regression lines. When P was less than 0·05 the two regression lines being compared were different significantly in slope.

Bliss (1935*b*) described a method to determine a factor, which he called the relative "potency", for any two insecticides whose data gave regression lines of the same slope. It was therefore impossible in our work to compare the "potencies" of insecticides whose regression lines lay in different groups, but it was possible to compute the relative "potencies" of those whose regression lines lay in the same group; and for this purpose a suitable standard for comparison was selected in each group: 3% β-butoxy-β′-thiocyanodiethylether for one and 0·4% pyrethrins for the other. The dosage-mortality data determined for each of these substances showed heterogeneity (see Table 1) so that the following formulae given by Bliss (1935*b*) were used:

$$M = \bar{x}_2 - x_1 + \left(\frac{y_1 - \bar{y}_2}{b_c}\right),$$

where antilog M is the relative potency. The accuracy with which M was determined was computed from

$$s_M = \frac{1}{b_c}\sqrt{\left(V(a_2) + V(a_1) + \frac{(\bar{y}_2 - \bar{y}_1)^2\, V(b_c)}{{b_c}^2}\right)},$$

and the standard error of the relative "potency" was given approximately by the following expression used by Cochran (Tattersfield & Martin, 1938):

$$\text{Standard error of relative potency} = \log_e 10 \times \text{antilog } M \times s_M.$$

In each group the relative potencies have been expressed as

$$\text{Relative potency} = \frac{\text{potency of the standard}}{\text{potency of the second curve}}.$$

The results of these computations are shown in Table 2.

TABLE 2. *Relative potencies of the insecticides used and results of tests for parallelism within the two groups*

$n = n_1 + n_2$, where n_1 and n_2 are the numbers of degrees of freedom in each of the regression lines. *P*..., Fisher (Table 4), 1936.

Insecticide		t_b	n	P	Relative potency
β-butoxy-β′-thiocyanodiethylether	3 %	—	—	—	Standard for Group I
" "	2 %	1·4029	50	> 0·1	2·038 ± 0·072
α-Naphthyl-isothiocyanate	3 %	1·8839	43	> 0·05	1·445 ± 0·074
Mixture { β-butoxy-β′-thiocyano-diethylether	1 %	1·334	44	> 0·1	2·083 ± 0·094
Mixture { Pyrethrins	0·1 %				
Pyrethrins	0·4 %	—	—	—	Standard for Group II
"	0·1 %	1·1904	27	> 0·2	3·068 ± 0·378
Dodecyl-thiocyanate	3 %	1·0885	24	> 0·2	4·537 ± 0·748

TABLE 3. *Dosage mortality data obtained by spraying eggs of bed-bugs of two different ages with 0·4 % pyrethrins in "odourless distillate". (See experiment H)*

Two batches Age of eggs in days	Wt./sq. cm. mg.	Log = dose	No. of eggs	Dead	% kill	Probit
2–5	0·15	1·18	71	28	39·5	4·73
	0·29	1·46	74	48	65·0	5·39
	0·54	1·73	48	40	83·5	5·97
0–3	0·15	1·18	65	26	40	4·75
	0·29	1·46	56	39	69·5	5·51
	0·54	1·73	58	51	88	6·17

DISCUSSION

The method elaborated by Bliss (1935*a*) and discussed by Tattersfield (1939) was used to express the results of our experiments because it appeared to be the most scientific method available, and enabled us to express our dosage-mortality data as straight lines whose accuracy could be calculated; to make comparisons between the sets of data with a known degree of accuracy; and to determine the reliability of the results.

The χ^2 test shows that in five of our dosage mortality curves the variation can be ascribed to the random sampling error. In the remaining three curves (Figs. 2, 6 and 9) the χ^2 test demonstrates a heterogeneity which cannot be fully explained.

Two factors that must have contributed to the heterogeneity were, first, the eggs were not individually randomized; secondly, there was evidence that the dosage administered was more variable than we had intended. In any future experiments with a Potter tower and light oil insecticides it will be necessary to measure the dose administered by weighing the deposit at the time of spraying. Preliminary experiments suggest that this would not be difficult.

Bliss (1935*a*) suggests that when the same toxic agent is used against a number of different population samples or different species of animals the variation in the slope of the regression lines is due to the variation in the susceptibility of the biological material; but when biological material in which there is little variation is treated with different toxic agents the variations in the slopes of the regression lines may be due (Bliss, 1939) to differences in the modes of action of the toxic substances. Although there must have been variations in our biological material, we think, in view of Exp. H on p. 258, and the number of eggs used in the course of the work, that the difference in slope of the two lots of regression lines in our work may be due to differences in the modes of action of the substances used. Some support is

given to this notion by the work of von Oettingen *et al.* (1936), who found that the mode of action of β-butoxy-β′-thiocyanodiethylether (= *n*-butyl carbitol rhodanate) on rats was different from that of lauryl-rhodanate; furthermore, from work of colleagues in this Department of the Imperial College it seems that β-butoxy-β′-thiocyanodiethylether and pyrethrins are different in their modes of action on insects.

Conclusions

3% β-butoxy-β′-thiocyanodiethylether in a kerosene oil is superior as an ovicide for eggs of the bed-bug, under laboratory conditions, to 3% α-naphthyl-isothiocyanate, 3% lauryl-rhodanate, 0·4% pyrethrins or a mixture of 1% β-butoxy-β′-thiocyanodiethylether and 0·1% pyrethrins, in a similar kerosene oil.

When the Potter spraying tower is used to test insecticides with a light oil base it is essential to maintain full control over the dosage administered by weighing the amount of spray deposited at the time of the experiment. A similar criticism probably applies to all types of testing apparatus.

The eggs of the bed-bug, *Cimex lectularius*, are unsuitable material for routine insecticide tests as they are very difficult to obtain in a condition suitable for randomization and their true age is sometimes doubtful.

Summary

1. The uses of liquid insecticides against the bed-bug are discussed.

2. Insecticide tests on eggs of *Cimex lectularius*, using a specially constructed spraying tower, are described in detail. An attempt made to administer an accurately known dosage of insecticide is described.

3. The results are expressed as regression lines obtained by Bliss's probit method and, where possible, as relative potencies: β-butoxy-β′-thiocyanodiethylether appears to be superior to the other compounds tested.

4. Evidence suggests that β-butoxy-β′-thiocyanodiethylether is different in its mode of action from lauryl-rhodanate and the pyrethrins.

Our thanks are due to Prof. J. W. Munro in whose Department this work was done.

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THE INSECTICIDAL PROPERTIES OF CERTAIN SPECIES OF *ANNONA* AND OF AN INDIAN STRAIN OF *MUNDULEA SERICEA* ("SUPLI")

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(With 1 Text-figure)

ANNONA SPECIES

Introduction

The family Annonaceae[1] or Anonaceae (custard-apple family) consists in the main of aromatic trees and shrubs of tropical origin. It contains from 800 to 900 species in some eighty genera, widely distributed in the tropics, with an extra-tropical ramification in Atlantic North America. Among the principal genera are *Asimina*, *Uvaria*, *Guatteria*, *Monodora*, *Rollinia*, *Xylopia*, *Annona* and *Artabotrys*. The genus *Annona* has about ninety species of trees and shrubs which are distributed mainly in tropical America, but a few are natives of the tropics of Asia and Africa. It is of undoubted economic importance, edible fruits such as cherimoya, custard-apple, sour sop, sweet sop are derived from certain species, and various parts of the plants of several of the species have some repute medicinally, for stupefying fish and for insecticidal purposes.

McIndoo & Sievers (1924) mention five species the seeds of which are reputed to be insecticidal: *A. cherimola* (reported by Greshoff, 1900, p. 12); *A. glabra*, pond apple (Maisch, 1885); *A. reticulata*, custard-apple (Maisch, 1885); *A. spinescens* (Greshoff, 1900, p. 12): Dragendorff (1898) states that the pulp of this species is also used as a fish poison and an insecticide; *A. squamosa*, sugar apple: Greshoff (1900, p. 11), quoting Hartwich, states that the seeds of the last-named are used against head lice, and Lyons (1907) reports that they are used as a parasiticide. Lindley (1846) states that "the leaves of *A. squamosa* have a heavy disagreeable odour and the seeds contain a highly acrid principle fatal to insects, on which account the natives of India use them powdered and mixed with the flour of Gram or *Cicer arietinum* for occasionally washing their hair".

Callan & Tutin (1911), quoting from *Pharm. Rev.* 1896, **14**, 231, say that the seeds of *A. muricata* and other closely allied species are employed for poisoning fish and for the extermination of destructive insects.

Chemistry of Annonaceae

The chemistry of plants of the family Annonaceae has been little investigated. Cortina (1901) is reported by Standley (1920–6) to have found among other constituents a resin present in *Annona cherimola* to which is ascribed the emetic-cathartic action of the seeds.

[1] Annonaceae is the correct spelling.

Callan & Tutin (1911) published an account of their chemical examination of the leaves of *Annona muricata*. They isolated a steam-volatile essential oil, a small amount of uncrystallizable alkaloid, several fatty acids, myricyl alcohol, sitosterol and a compound "anonol", $C_{23}H_{38}O_4$, which was later regarded by Power & Salway (1913) as being a member of the group of phytosterol glucosides (phytosterolines).

Trimurti (1924) isolated a minute amount of an alkaloid from the leaves of *A. squamosa*, but glucosides were not shown to be present. Santos (1930) obtained an alkaloid, anonaine, $C_{17}H_{16}O_3N$, in a yield of 0·03–0·04% from the bark of *A. reticulata*, M.P. 122–123° C.; $[\alpha]_D^{32·5°} - 83·0°$ (chloroform). The same alkaloid was isolated from the bark of *A. squamosa* by Reyes & Santos (1931), in a yield of 0·14%, but a slightly different formula was later ascribed to it, $C_{17}H_{17}O_3N$ (Santos, 1932). From the bark of the closely allied genus *Asimina*, sp. *triloba*, Manske (1938) isolated the alkaloid anolobine, $C_{17}H_{17}O_3N$. Santos & Reyes (1932) and Barger & Sargent (1939) made studies of the alkaloids of *Artabotrys suaveolens*, N.O. Annonaceae, and the three alkaloids isolated from the bark by the latter investigators were:

(1) Artabotrine	0·19%	$C_{20}H_{23}O_4N$
(2) Suaveoline	0·0013%	$C_{19}H_{21}O_4N$
(3) Artabotrinine	0·012%	$C_{18}H_{17}O_3N$

Barger & Sargent consider artabotrine probably to be 10-hydroxy-4:5:6-trimethoxyaporphine; suaveoline, 4:10-dihydroxy-5:6-dimethoxyaporphine; and artabotrinine, 2-methoxy-5:6-methylenedioxy*nor*aporphine.

Insecticidal properties of Annona *spp.*

In 1938 a report of the insecticidal effect of *Annona reticulata* upon the scale insect, *Lecanium viride* Green, was issued by the Mysore Department of Agriculture (1938). Hot alcoholic extracts, water suspensions of the concentrated hot alcoholic extracts of seed and apparently alcoholic extracts of stem, bark and leaf and root bark were tested. All were found to be toxic: "the chemically prepared alcoholic extract of the seed was very toxic at 0·125% strength, a mortality of 70–80% being obtained". It is, however, not quite clear whether the concentration is expressed in terms of the plant material or the extract. Extracts of stem bark, leaf and root bark at 10% concentration gave respectively 100, 70 and 60% mortalities. No information is available as to the method of testing employed, but it was claimed that to this insect the hot alcoholic extracts of the seed of *Annona reticulata* were more toxic than *Derris elliptica*. As, however, no particulars were given of the analytical characteristics of the derris used this comparison may possess little value. Tests with other plants including *Mundulea suberosa* (*sericea*) were carried out. Extracts of none of them approached those of the seed of *Annona reticulata* in potency.

In view of these results a supply of several species of *Annona* was obtained from Ceylon through the good offices of the Colonial Office and the Department of Agriculture, Peradeniya, Ceylon. They were *A. reticulata* (custard-apple), leaves, stem, root and seed; *A. palustris* (Glabra) (alligator apple), leaves, stem, root; *A. muricata* (sour sop), leaves, stem, root, seed; *A. squamosa* (sugar apple), leaves, stem, root. Where omitted the seed was not available at the time of collection.

Experimental

Insecticide tests

A sorting-out series of insecticidal tests was carried out by extracting 10 g. of each of the samples with absolute alcohol in the cold. These extracts were made up to a strength of 20% in terms of part of the plant, and the appropriate dilutions to be tested prepared by diluting with 0·5% saponin solution in water. In these trials the amount of alcohol was not adjusted to the same concentration for each dilution tested, but for the corresponding dilutions the concentration of alcohol was always the same. This was of importance, since the chief comparisons to be made were between the corresponding dilutions of each part of the plants.

Table 1. *Biological trials with* Annona *species. Insect used:* Macrosiphoniella sanborni (*chrysanthemum aphis*)

Alcoholic extracts diluted with 0·5% saponin solution in water

Part of plant	Conc. % part of plant	% paralysed	Part of plant	Conc. % part of plant	% paralysed
		Annona reticulata			
Seed	5·0	100	Leaf	5·0	100
	1·0	100		1·0	100
	0·2	90		0·2	50
Stem	5·0	100	Root	5·0	100
	1·0	100		1·0	95
	0·2	27		0·2	90
		Annona muricata			
Seed	5·0	95	Leaf	5·0	50
	1·0	70		1·0	5
	0·2	5		0·2	0
Stem	5·0	No	Root	5·0	43
	1·0	material		1·0	16
	0·2	toxicity		0·2	0
		Annona squamosa			
Stem	5·0	No	Leaf	5·0	100
	1·0	material		1·0	95
	0·2	toxicity		0·2	43
			Root	5·0	35
				1·0	18
				0·2	5
		Annona palustris			
Stem	5·0	No	Leaf	5·0	100
	1·0	material		1·0	62
	0·2	toxicity		0·2	0
			Root	5·0	No material toxicity
				1·0	
				0·2	
		Mundulea sericea ("Supli") (from West Nasik, India), 1938 sample			
Bark	5·0	100	Leaf	5·0	100
	1·0	95		1·0	100
	0·2	95		0·2	68
Debarked Stem	5·0	100	Root	5·0	100
	1·0	100		1·0	100
	0·2	5		0·2	100
Control Alcohol		0			
saponin solutions		0			
		0			

A sample of *Mundulea sericea* ("Supli"), received in 1938 from India, was included in the tests for purposes of comparison. The tests were carried out in the Tattersfield-Morris spraying apparatus (1924, 1934), the test-subject being *Macrosiphoniella sanborni* Gill., the chrysanthemum aphis, and twenty insects were used for each test. The dilutions were rather widely spaced.

The results (Table 1) indicate that among the *Annona* species tested the leaf has in every case a measure of toxicity to this insect, but that of *A. muricata* leaf is very slight. The stem and root of *A. reticulata* alone have any material toxicity. Only two samples of seed were included. Those of *A. reticulata* were pronouncedly toxic, those of *A. muricata* possessed some insecticidal activity but not to such an extent as to make this plant of any real interest as an insecticide. The toxic properties of the leaf of *A. squamosa* indicate that the seed of this plant would be worth investigation.

A. reticulata is the most toxic of the four species of *Annona*, and it is worth further study. The results, however, obtained on the same day with the several parts of the *Mundulea sericea* plant show that none of the parts of any of *Annona* species possesses any superiority in toxicity to the bark and root of this plant, the leaves of which are also insecticidal.

Further tests were carried out with *A. reticulata* on a wild colony of *Aphis rumicis* reared in the open. The margin between the concentrations was narrowed and an alcoholic extract of a rich *Derris elliptica* Changi no. 3 was included for purposes of comparison. The alcoholic extracts of the several parts of *Annona reticulata* were prepared as before and diluted with 0·5 % saponin solution. This attempt to bring the concentrations nearer together and the use of a wild colony of aphides introduced heterogeneity into the data in the case of the seed and leaf and to a less extent of the root, but with the sample of derris the results, when plotted in probit-log concentration form, were closely linear and a straight line could be fitted by eye to the data. It is impossible to say whether the effect with the *Annona* species is due to a great excess of matter of low toxicity or to the slow toxic action of the active principles. The insecticidal action is definitely of a delayed type, which tends to increase the difficulty of biological evaluation. Several hours elapsed in our trials before the onset of paralysis, which with aphides was slowly accentuated, and in the meanwhile reproduction may take place. This also happens to some extent with *Derris elliptica*. Accurate comparisons of the relative toxicities of the different parts of *Annona reticulata* and *Derris elliptica* to *Aphis rumicis* could not be made but the approximate median lethal concentrations in terms of part of plant in g./100 c.c. are given in Table 2. The roughly approximate concentrations giving 90–100 % mortalities are also given for purposes of comparison.

TABLE 2. *Toxicity to* Aphis rumicis *of alcoholic extracts of* Annona reticulata

(Insects sprayed in fivefold replication, 10 at a time)

Name	Part of plant	% part of plant causing 50 % mortality	% part of plant causing 90–100 % mortality
Annona reticulata	Seed	0·1–0·15	0·25
,, ,,	Leaf	0·2–0·25	0·5–1·0
,, ,,	Stem	Little toxicity at 0·75	
,, ,,	Root	0·1–0·2	Less than 0·5
Derris elliptica, Changi type no. 3	Root	0·01	0·02

It is thus seen that the rich root of *Derris elliptica* (rotenone content approximately 9% and ether extract 25–27% on dry-matter basis) is decidedly more toxic to *Aphis rumicis* than any part of *Annona reticulata*.

Relative potencies of the ether extract of the seed of A. reticulata, *nicotine and artabotrine base.*

It is shown above that with an insecticide having a markedly delayed action there is some difficulty in obtaining statistically homogeneous data. Trials were carried out using carefully randomized insectary-reared *Macrosiphoniella sanborni* (chrysanthemum aphis) and an ether extract of the seed of *Annona reticulata*. 10 g. of the ground seed were extracted for several hours with ether, the partially extracted seed reground and subjected to a further exhaustive extraction, the bulk of the ether taken off on a water-bath and finally *in vacuo* to constant weight. The yield of extract amounted to 33·8% of the seed. It was dissolved in slightly warm alcohol diluted with 0·5% aqueous saponin solution, each dilution being brought to a constant percentage of 10% by volume of alcohol. Nicotine (pure) was also diluted in a similar way and sprayed for comparative purposes. After spraying the insects were set aside in a cellar of approximately constant temperature and examined after 20 and 44 hr. The results are given in Table 3, and graphed in probit-log concentration form in Fig. 1.

TABLE 3. *Toxicities of the ether extract of the seed of* A. reticulata, *nicotine and artabotrine. Test insect:* Macrosiphoniella sanborni

(Sprayed in fivefold replication—ten insects at a time. Date 9 October 1939)

Substance and mode of application	Conc. (mg./100 c.c.) in terms of part of plant	Conc. (mg./100 c.c.) in terms of part of extract	% of insects paralysed: After 20 hr.	% of insects paralysed: After 44 hr. allowing for control
A. reticulata seed. Alcohol solution of extract in aqueous saponin solution	300	100	91·3	95·5
	200	68	81·1	94·4
	150	51	71·1	88·4
	100	34	54·2	80·8
	75	25	40	78·7
	50	17	7·8	18·7
		Conc. mg./100 c.c.		
Nicotine. Alcohol solution in aqueous saponin solution	—	60	100	100
	—	40	98	95·6
		20	87·8	74·5
		10	62·3	45·9
		5	20·8	9·8
Artabotrine base	—	—	Not materially toxic at concentration of 60 mg./100 c.c.	
Controls: alcohol 10 c.c./100 c.c. saponin 0·5 g./100 c.c.	—	—	0	4·1
	9 November 1939			
A. reticulata seed. Acetone solution of extract in aqueous saponin solution	—	60·2	72	Heterogeneity in the data
	—	47·9	59·2	
	—	38·0	44	
		30·2	37·5	
		26·9	21·6	
		24	2·1	
		21·4	16·7	
		19	8·5	
Controls (4-fold replication): acetone 10 c.c./100 c.c. saponin 0·5 g./100 c.c.	—	—	0	

Tests were also carried out at the same time on *Artabotrine* base, one of the alkaloids of *Artabotrys suaveolens*, a genus closely allied to *Annona*. We are greatly indebted to Mr J. Sargent for a gift of this substance. It was worked up for spraying by dissolving in hot alcohol, cooling, diluting as before, and was tested at the same concentrations as nicotine. It did not prove materially toxic at these concentrations.

Table 3 and Fig. 1 demonstrate very clearly the delayed action of the extracts of the seed of *Annona reticulata*, for whereas on the second day's examination in the case of the nicotine

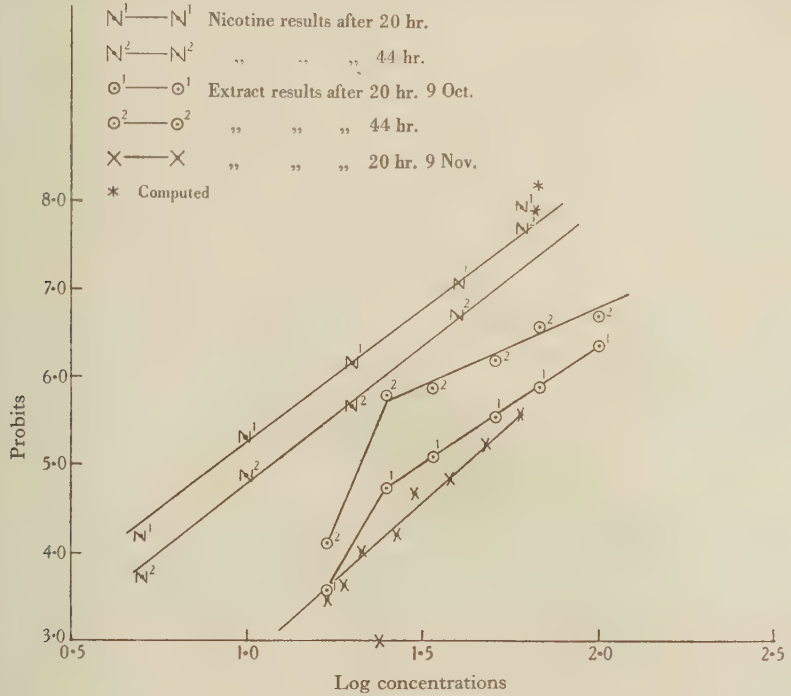

Fig. 1. Toxicity of ether extract of *Annona reticulata* and of nicotine to *Macrosiphoniella sanborni*.

there are at every concentration, except the highest, a proportion of recoveries, at all concentrations of the extract the toxic effect has increased with time, the increase being proportionally greater as the concentration declines. With the exception of the lowest concentrations a straight line can be drawn through the points when plotted in probit-log concentration form. A statistical analysis (χ^2 test) shows that in each case there is no significant departure from homogeneity over the straight line range, but that the regression line for the second day's results for the extract departs significantly from parallelism with the others. Since also over the straight line range for the second day, the median lethal dose is not reached in the case of the extract, comparisons of the toxicities for the second day could not be estimated with accuracy. The relative potencies were as follows:

	Calculated	Estimated from graph (median lethal concentration)
Nicotine 1st day : Nicotine 2nd day	$1{\cdot}36 \pm 0{\cdot}15 : 1$	1·39 : 1
Nicotine 1st day : Extract 1st day	$3{\cdot}87 \pm 0{\cdot}42 : 1$	3·9 : 1

As there is much reproduction of living young during the second day in each case, it is doubtful whether a comparison of the second day's results would have any practical meaning, but the potency of nicotine (2nd day) relative to the extract (1st day) is approximately 2·8. There is much oil present in the extract, and there is a possibility that this may itself not only possess toxicity, but also accentuate that of the active principle. Nevertheless, it is fairly clear that the active principle must have a relatively high insecticidal potency to aphides.

It will be noted from Table 3 and Fig. 1 that in the case of the extract of the seed of *A. reticulata* there is a steep decline in toxic action to the lowest concentration tested. It frequently happens in tests of this nature that breaks occur in the probit-log concentration regression lines at the lower concentrations, but such breaks are usually in the reverse direction from that shown in the present case, and since they have little practical bearing, it has been our practice to discard these lower aberrant values in calculating the results. In order to ascertain whether in this case the sudden fall in toxic value was a chance result arising from imperfect random sampling, some error in technique or the use of too few insects for the estimate of such a low kill, the experiment was repeated using a fresh ether extract and both alcohol and acetone as solvents. The alcoholic solutions gave heterogeneous data for both the first and second day's results which were discarded. The acetone solution diluted as before gave homogeneous data for the first day's results if one value (at a concentration of 24 mg./100 c.c.) was discarded. The results are given in Table 3. The sudden fall in toxic values at 24 mg./100 c.c. is to be noted; it can be compared with a similar fall at 17 mg./100 c.c. in the earlier test on 9 October. At this stage, however, it can only be regarded as fortuitous, since still lower concentrations gave higher values falling near to the regression line.

Although a strict comparison could not be made between the data obtained for the extracts for the two dates separated by a month, it was of interest to ascertain in what direction, if any, the relative toxicity had changed. The regression lines (results after 20 hr.) showed a departure from parallelism that was almost, but not quite, significant ($\chi^2 = 3{\cdot}334$). The slope of the regression line on the second occasion is somewhat greater than on the first.

The potency of the extract on 9 October relative to that tested on 9 November was as 1·3 : 1 compared at the median lethal concentration. A comparison, however, at concentrations giving high percentage effects would not indicate much difference in potency between the two occasions, and the less steep decline of the regression line on the earlier date may be due in some obscure way to the cumulative effect of the poison. The second set of results confirm the earlier ones as to the relatively high potency of the active principle.

Tests with insects other than aphides.

A few tests were carried out with adult *Oryzaephilus surinamensis* L., the saw-toothed grain beetle, an insect probably more resistant to the action of insecticides than those used in the previous trials. A new form of atomizing apparatus was also used which gave a much lighter deposit and the higher concentrations required to secure an effective mortality were undoubtedly partly due to this cause. The tests included the roots of *Annona reticulata*, *Mundulea sericea* ("Supli") from India, and *Derris elliptica* (Changi no. 3 of the same quality as above). The results are given in Table 4.

The root of *Annona reticulata* is shown to be much less toxic to this insect than the roots of either *Mundulea sericea* or of *Derris elliptica* (Changi 3).

TABLE 4. *Toxicities of alcoholic extracts of three fish-poison plants to* Oryzaephilus surinamensis

(Preliminary trials)

Part of plant	Conc. % part of plant	% paralysed
Annona reticulata, root	2·5	14·5
Mundulea sericea, root "Supli"	2·5	86·3
Derris elliptica, Changi no. 3, root	0·25	96
Controls: 2·5 % *w/v* alcohol, 0·5 % *w/v* saponin	—	3

Stomach-poison trial.

A preliminary trial in which nearly fully grown larvae of *Orgyia antiqua* (vapourer moth) were fed upon apple foliage which had been sprayed with the alcoholic extract of the seed of *Annona reticulata*, diluted with saponin solution, did not show any deterrent or stomach-poison effect. The insects fed as freely as in the unsprayed controls.

CHEMICAL INVESTIGATION

The samples were too small to permit of any serious attempt to isolate the active principles. A few preliminary tests showed that toxic material was extracted by ether and alcohol, but owing to the presence of much oil in the seed and the consequent difficulty in grinding to a fine state of division, extraction may be complete only after a prolonged period. In the case of the leaf the alcohol extract after ether extraction showed no toxicity to the chrysanthemum aphis (*Macrosiphoniella sanborni*).

The alcohol and ether extracts of the seed were combined, dissolved in ether and extracted with 10 % hydrochloric acid. The hydrochloric acid extract was ammonified, extracted with ether and evaporated. The residue showed little if any loss of toxicity, but the hydrochloric acid extract was slightly toxic, and this was also true when ether extracts of the leaf were similarly treated. The powdered root was percolated with cold alcohol, the alcohol taken off under reduced pressure, the residue macerated with saturated sodium carbonate solution and the latter extracted by ether. Any toxic material from the ether solution was extracted by hydrochloric acid which after ammonification and ether extraction gave a small oily residue possessing a characteristic odour which, however, was not toxic to insects at a concentration of 0·04 %. Durham tests for rotenone-like substances were negative with all the extracts; a slight, fugitive and rather indefinite purple colour was given with the extract of the root with fuming nitric acid, which was, however, discharged on adding ammonia.

MUNDULEA SERICEA (WILLD) (=*SUBEROSA* BENTH.)

INTRODUCTION

The botany, ecology and distribution of this plant have been reviewed by Greenway (1936). The genus *Mundulea* contains 20 species, the majority of which are recorded from Madagascar. At one time included under *Tephrosia*, it is now regarded as a connecting link between that genus and *Millettia*. *Mundulea sericea* has been found in India, Ceylon, Madagascar and in Africa from the Sudan to Natal. The seeds and bark have been used in

India and the bark in East Africa for poisoning fish. An erect shrub or a slender tree up to 25 ft. tall, it shows either a smooth, greenish yellow-brown bark, or a very rough longitudinally fissured, very corky yellowish brown bark. Greenway states that in his opinion no satisfactory botanical distinctions can be made between the types having smooth and rough barks. The former occurs under forest formations, and the latter in open bush or savannah areas which are subject to fire.

Insecticidal properties

The insecticidal properties of this plant were tested in a preliminary way by Tattersfield & Gimingham (1932). Of two samples, one from South Africa proved non-toxic, whereas a sample from India was found to possess stems, seeds and pods which had insecticidal properties. Worsley (1936, 1937) examined specimens of *M. sericea* (*suberosa*), naturally occurring in Tanganyika, and found certain of them to possess relatively high insecticidal values. He detected rotenone in the bark and estimated its amount by a special technique. He found, however, that of the two types, one possessing smooth and the other a corky bark, those having a corky bark had little or no rotenone and their toxicity was of a low order as contrasted with those with a smooth bark. Worsley considered that these differences were due, either to varietal differences, or to periodic burning resulting in the corky texture of the bark and loss of insecticidal potency.

Worsley found that the most toxic parts were the bark and seed, the leaf, twigs and roots of his sample possessing little or no insecticidal activity. A sample, however, of South African root tested at Rothamsted in 1934 showed some toxicity to *Aphis rumicis*.

In 1936 we received a specimen of the leaves of *Mundulea sericea* from the Conservator of Forests, Nasik, Bombay Presidency, India, which proved to be toxic to *Aphis rumicis*. In view of this result a further sample was asked for and leaves, bark, root and debarked stem of "Supli" (*Mundulea sericea*) were received in 1938 from West Nasik, India. Dr F. N. Howes kindly compared the leaves with material in the Herbarium at the Royal Botanic Gardens, Kew, and found them to agree with those of *M. sericea* (Wiild.) from different parts of India. Howes (*in lit.*) says "that like most plants that have a wide range and have been long in cultivation or 'domesticated' this species is a variable one, particularly in leaf characters". It would thus be very easy to assume, without apparent justification, from the leaf form that the specimens of this plant from India and Africa were botanically of different species. In our tests upon the chrysanthemum aphis (*Macrosiphoniella sanborni*) leaves, bark, root and debarked stem were found toxic. Subramaniam (1934) also observed that grain was protected against Bruchids by dusting with the powdered root bark of *Mundulea sericea*. It would appear, therefore, that the specimens of this plant grown in India contain insecticidal principles in other parts than the stem bark, the most potent portion of the plant in African specimens so far examined. Whether this is due to differences in variety, climate or soil is not clear.

Experimental

Insecticide tests

In Table 1 are given the results of preliminary laboratory insecticidal tests carried out on various parts of *Mundulea sericea* at the same time as those made on the *Annona* species. It is obvious from these results that all parts have relatively high potency to the aphis *Macro-*

siphoniella sanborni. Preliminary trials using adult *Oryzaephilus surinamensis* (Table 4) indicate that the root of *Mundulea sericea* has a measure of toxicity to this beetle, but that a *Derris* root, of the rich strain Changi no. 3, is a much more powerful insecticide.

TABLE 5. *Toxicity of different parts of* Mundulea sericea (*"Supli"*).
Test subject: Oryzaephilus surinamensis (*fivefold replications*)

1st series. Time of spraying, 17½ sec. Range of deposit, 1·80 mg.–2·08 mg./cm.² 2 August 1939.

Concentration part of plant g./100 c.c.	% paralysed after 18 hr. allowing for control		
	Leaf	Bark	Root
3·0	100	—*	97·6
2·0	74	68	100
1·0	24·3	23·3	55·8
0·5	7·2	2·3	21·6
0·25	—	—	9·7
0·1	—	—	4·5
Control (mean of 6 tests)	3·7	—	—

* This result was completely out of line with others and the tests were repeated (see below).

2nd series. Time of spraying, 16–17 sec. Range of deposit, 2·5–2·8 mg./cm.² Results after 2 days. 9 August 1939.

3·0	—	98·1	—
2·0	—	81·2	96·4
1·5	—	64·3	94·3
1·0	—	27·2	70·6
0·5	—	—	50·1
Control (mean of 2 tests)	1·9	—	—

A further series of trials was made using adult *Oryzaephilus surinamensis* L., in order to compare the relative potencies of the alcoholic extracts of the several parts of the plant. The data are given in Table 5. Leaf, bark and root show a fairly high degree of insecticidal activity. The debarked stem was only slightly toxic at a concentration of 5 per cent. In the first series of tests given in Table 5 the bark is shown to be of the same order of toxicity as the leaf and rather less toxic than the root. A repetition 7 days later of trials on the bark and root confirmed the greater insecticidal potency of the root.

If the published insecticidal results of the African strain are representative, it is clear that the above sample of *Mundulea sericea* from India differs from the African strain in the greater toxicity of leaf and root.

Durham tests on extracts of leaf, bark, stem and root were negative with all parts of the plant except the root. The microtechnique of Worsley (1936, 1937) was not carried out, and it is possible that extractable substances other than rotenoids hid the characteristic coloration except in the case of the root. Attempts failed, however, to separate rotenone from the root.

SUMMARY

1. Laboratory insecticidal trials, made with several species of *Annona*, *A. muricata*, *A. palustris*, *A. reticulata*, *A. squamosa*, show that plants of this genus possess contact insecticidal properties to aphides. *A. reticulata* was the most potent of those tested. Seed, root, leaf and to a less extent the stem of this species possessed contact insecticidal properties. The seed of *A. muricata* and the leaves of *A. squamosa* and *palustris* also showed contact toxicity, but were not so potent as *A. reticulata*. The seed of *A. squamosa* was not available for testing purposes.

2. None of the above are superior to the leaf, root and bark of *Mundulea sericea* Wiild. of the variety known in India as "Supli", and are much less toxic than the richer specimens of *Derris elliptica* root such as Changi no. 3.

3. The toxic action of the *Annona* spp. appears to be rather specific. Aphides are susceptible, but adult *Oryzaephilus surinamensis* L., which are susceptible to the rotenone class of insecticides, are resistant.

4. Tests made with extracts of a variety of *Mundulea sericea* Wiild. from India showed that leaf, bark and root possessed marked insecticidal properties when tested on *Macrosiphoniella sanborni* and *Oryzaephilus surinamensis*. This is in contrast with the African variety, the leaf and root of which are reported as distinctly less potent than the bark. The toxicity of none of these parts is of the same order as that of the root of *Derris elliptica*, Changi no. 3.

We are indebted to the Colonial Office and the Director of Agriculture in Ceylon for the supply of *Annona* species, to the Conservator of Forests, Northern Circle, Bombay Presidency, India, for a supply of *Mundulea sericea*, and to the Ministry of Agriculture and the Colonial Development Fund for grants towards this work. Our thanks are also due to Dr F. N. Howes, Royal Botanic Gardens, Kew, for material help on the botanical side of this work, to Mr L. J. Sargent for gifts of artabotrine base and hydrochloride and to Drs J. T. Martin and S. H. Harper for assistance during the course of the investigation.

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THE PROBLEM OF THE EVALUATION OF ROTENONE-CONTAINING PLANTS

V. THE RELATIVE TOXICITIES OF DIFFERENT SPECIES OF DERRIS

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(With 1 Text-figure)

Two species of derris are of economic importance as insecticides, *Derris malaccensis* and *D. elliptica*. Included in each species are a number of varieties or races, of which the botanical characteristics have been discussed by Henderson (1934). Much admirable selection work by Georgi and his associates in Malaya has resulted in the development of a variety of *D. elliptica*, known as Changi no. 3, showing a rotenone content of the order of 12% and of ether extractives of the order of 25–30% (Georgi, 1939). Varieties of *D. malaccensis* are characterized by the presence in their resins of varying quantities of toxicarol and, in general, by a low proportion of rotenone to total ether extractives. The roots of *D. elliptica* contain little or no toxicarol.

Of the active principles so far isolated, rotenone has proved to be the most toxic to insects, but the rotenone-free resin plays an important part in the toxic action of the root, and other active principles have recently been isolated. Martin & Tattersfield (1936; see also Tattersfield & Martin, 1937) have separated from a variety of *D. malaccensis* naturally occurring toxicarol (15-hydroxy-deguelin), shown by Cahn *et al.* (1938*a*) to be *l*-α-toxicarol. Cahn & Boam (1935) have separated sumatrol, or 15-hydroxy-rotenone, while Meyer & Koolhaas (1939) and Harper (1939) have independently obtained the active precursor, termed by Harper "elliptone", of the compound isolated by Buckley (1936). Meyer & Koolhaas (1939) have also obtained malaccol, shown by Harper (1940) to be 15-hydroxy-elliptone. Naturally occurring deguelin has yet to be isolated. We have shown (Tattersfield & Martin, 1938) that, to the bean aphis, *Aphis rumicis* L., rotenone is 4–6 times more toxic than preparations of rotenone- and toxicarol-free derris resins, and some 13–15 times more toxic than either *l*-α-toxicarol or sumatrol. Cahn *et al.* (1938*b*) have suggested that to adults of the beetle, *Ahasverus advena* Waltl., sumatrol has a toxicity approaching that of rotenone.

It is generally accepted that while the content of rotenone or of ether extractives may be relied upon to evaluate roots within a given species, they cannot be used separately to evaluate all types of derris, and in trading, the practice has been to assess the root on its rotenone content, ether extract, or on the contents of rotenone and ether extractives combined.

Several methods of chemical evaluation of derris and other rotenone-containing plants have been suggested. These have sought to make adequate allowances for the additional toxic effect of the non-rotenone fraction of the resin. We have found (Tattersfield & Martin, 1935) that with derris roots of the same species, the determinations of rotenone, ether

extract or methoxyl content gave fairly close measures of their insecticidal activities, but wide discrepancies were encountered when roots of different species were compared. The summation of the percentages of the rotenone and the dehydro compounds separated from the residual resin gave a much more satisfactory evaluation of the toxicities of different species. This degree of agreement is probably accounted for by the fact that, while toxicarol is much less toxic than the deguelin fraction, the separation of dehydrotoxicarol by the method used was far from quantitative. Jones *et al.* (1935) found in tests with derris, cube and tephrosia on houseflies, that the methoxyl values of the extracts, corrected for the toxicarol present, gave better agreements with toxicities than did other determinations.

The naturally occurring active principles are optically active, and polarimetric methods have therefore been used as a means of evaluation. There is, however, no strict correlation between the toxicities of the active principles and their rotatory powers. We have found that to *Aphis rumicis* rotenone is some 13–15 times more toxic than is the toxicarol precursor, while its specific rotation in benzene is approximately three times greater than that of the active toxicarol. Preliminary trials here have shown that *l*-elliptone, with a specific rotation in benzene of $-19°$, is approximately one-third as toxic as rotenone, of specific rotation $-240°$, to adult *Oryzaephilus surinamensis* L., when tested in benzene emulsions. Jones (1936), in a study of the rotatory powers of extracts of derris and cube in relation to toxicity to houseflies, found that the combined optical rotatory powers of acetone and benzene extracts gave an approximate measure of insecticidal effectiveness. He concluded, however, that the use of optical rotatory power could not be recommended in preference to an alternative method based upon the rotenone and total extractive contents. We have found (Tattersfield & Martin, 1936) that the specific rotations of the resins in benzene have placed particular samples of derris in their correct *order* of biological activity, without showing with any degree of accuracy the relative toxicities of the roots to *Aphis rumicis*. Worsley (1937*a*, *b*) has suggested for evaluation purposes the determination of the "toxicity ratio", equal to the reciprocal of the percentage of optical constituents present in the root of derris or bark of *Mundulea*. This he states will be directly proportional to the weight of derris or *Mundulea* required to gave equal toxic effects. Jones & Smith (1936) have made an allowance for the non-rotenone fraction of the resin by assigning to it a definite toxic value. The toxicity of derris root to houseflies is assessed by adding to the rotenone content one-half of the percentage of residual resin in the case of benzene or carbon tetrachloride extraction, or by the addition of two-fifths of the rotenone-free resin when acetone is used as extractant.

The different species and varieties of derris vary considerably in the relative proportions of the active principles present. In view of the widely differing toxicities of the active principles, it is a matter of importance to know how derris species compare in toxicity, and whether, knowing the relative toxicities and amounts of the active principles present, it is possible chemically to evaluate the insecticidal potencies of different roots.

We have therefore carried out biological and chemical tests upon derris roots kindly supplied to us by the Director of Agriculture, Straits Settlements and Federated Malay States.

Experimental

Description of the roots. Four types of root were received, in quantities of the order of 0·5 cwt., in the chopped condition. They were:

D. malaccensis, Kinta (our reference no. W. 211).
„ var. Sarawakensis (our reference no. W. 212).
D. elliptica, Sarawak creeping (our reference no. W. 213).
„ Changi no. 3 (our reference no. W. 214).

The roots were coarsely ground for us by Messrs British Drug Houses, Ltd., and were then reground by us using a C. and N. Laboratory mill with a fine copper gauze screen.

In order to ascertain the relative degrees of fineness of the roots, sieving tests were carried out on 25 g. portions using 60- and 100-mesh woven wire standard sieves as appointed by the Institute of Mining and Metallurgy. The results were as follows:

	Percentage of root		
	Retained on 60-mesh sieve	Passed 60-mesh but retained on 100-mesh sieve	Passed 100-mesh sieve
D. malaccensis, Kinta (W. 211)	0·8	18·4	80·8
„ var. Sarawakensis (W. 212)	1·2	19·2	79·6
D. elliptica, Sarawak creeping (W. 213)	7·6	26·0	66·4
„ Changi (W. 214)	7·5	19·5	73·0

The sieves were shaken by hand, with occasional tapping. The sieving method of determination of the fineness of ground derris root has, in our experience, proved unsatisfactory. A fraction of the ground root consists of spicular particles, and these, on continued agitation of the sieve, tend to pass through the meshes longitudinally. It is possible that one worker may obtain comparable results, but the values obtained by different workers are likely to differ widely. The figures quoted, however, give an indication of the relative degrees of fineness of the roots under examination.

The nature of the fibrous fraction of the root. The fraction of the root difficult to reduce to a fine powder on grinding, and appearing as "fluffy" material, was investigated. Small whole pieces of the air-dried *D. elliptica*, Changi root, were soaked in water for some days. Transverse and longitudinal sections were then prepared, and suitably stained. Sclerenchymatous fibres were seen in association with both phloem and xylem tissue. On grinding, the fibres appeared in the coarse fractions, with narrow annular and spiral vessels attached. Tested by Durham's reagent the fibres were rotenone-free, but the associated tissues gave a positive Durham test. On regrinding and resieving the coarse fraction, the tissues associated with the fibres were removed, and the rotenone-free sclerenchymatous fibres, the "fluffy" material proper, remained.

Biological investigations

Aphis rumicis, obtained from broad bean plants, or on one occasion from thistles, has been used as the main test subject in the investigations to be reported. The adult apterous insects were selected and placed in tubes of ten insects each during the morning before spraying. They were sprayed on flannelette disks in the apparatus described by Tattersfield (1934), and after spraying were placed, on the disks, in tubes with a small amount of food supply, and kept in a dark room at a fairly constant temperature of about 16° C.

The insects were examined for toxic effects 18–24 hr. later, and again on the second day after spraying. In assessing the mortalities, it has been our practice to separate the insects into the categories:

Normal insects (*N*).

Slightly affected insects (*S*), those able to walk but showing symptoms of paralysis such as a hesitant movement.

Badly affected insects (*B*), those not able to walk more than a few steps before falling over or exhibiting convulsive movements.

Moribund insects (*M*), those only able to move appendages, and the apparently dead insects (*D*).

With a quickly acting poison such as pyrethrum, in which the effect tends to be evanescent, the badly affected insects may show a tendency to recovery with time, but with the more slowly acting derris poisons the effect on the badly affected insects almost invariably deepens. In the work to be reported we have therefore taken the percentages of badly affected, moribund and dead insects ($B+M+D$) in assessing the results. This method in our hands has proved the most suitable mode of assessment. Since the degree of paralysis may vary in different cases there would seem to be the need for a physiological method of determining the intensity of effect or some statistically sound system of awarding marks.

The relative toxicities of the derris roots to Aphis rumicis

In the first tests, quantities of the order of 1·0–2·5 g. of the *Derris malaccensis*, Kinta (W. 211), *D. malaccensis*, var. Sarawakensis (W. 212) and the *D. elliptica*, Changi (W. 214) roots, were extracted in the Soxhlet apparatus with ether for a period of 6 hr. The solvent was removed, finally under reduced pressure, the resins dissolved in absolute alcohol and made up to definite volumes. Decreasing aliquots of each were added, immediately before spraying, to 0·5 % saponin solution and the alcohol contents adjusted to 5 %. The results of the examination on the second day after spraying are given in Table 1, and the corresponding regression lines, obtained by plotting the logarithms of the concentrations of root against the probit values (Bliss, 1935), in Fig. 1A.

The *D. malaccensis*, var. Sarawakensis root is seen to be intermediate in toxicity between the *D. elliptica*, Changi, and the *D. malaccensis*, Kinta roots.

Further tests with *Aphis rumicis* were carried out as before, using the *Derris malaccensis*, Kinta (W. 211), *D. malaccensis*, var. Sarawakensis (W. 212) and *D. elliptica*, Sarawak creeping (W. 213) roots. The results of examination one day after spraying are given in Table 2, and the probit values in Fig. 1B.

The *D. malaccensis*, var. Sarawakensis root, again shows an appreciably greater toxicity than the *D. malaccensis*, Kinta root. The *D. elliptica*, Sarawak creeping root, shows a toxicity less than, but approaching that of the *D. malaccensis*, var. Sarawakensis root.

The toxicities to *Aphis rumicis* of the *Derris malaccensis*, Kinta and *D. elliptica*, Sarawak creeping roots were then compared with that of rotenone. In this case, the resins were extracted from the roots by percolation with ethyl acetate, the solvent being removed by warming under reduced pressure. The resins were then dissolved in a little ether which in turn was removed by distillation followed by warming under reduced pressure. Weighed quantities of the resins were dissolved in alcohol before dilution. The results are given in Table 3 and in Fig. 1C.

A great divergence is seen between the toxicities of rotenone and the roots, but the *D. elliptica*, Sarawak creeping root, again shows a slightly greater toxicity than that of the *D. malaccensis*, Kinta root.

The relative toxicities of the roots and rotenone, determined from the freehand probit-log concentration lines in Fig. 1, were as follows:

TABLE 1. *Comparison of the toxicities of* D. malaccensis, *Kinta* (*W.* 211), D. malaccensis, *var. Sarawakensis* (*W.* 212) *and* D. elliptica, *Changi* (*W.* 214) *roots, to* Aphis rumicis *from broad beans. Tests of* 15 *June* 1938. *Fivefold replication of* 10 *insects. Results two days after spraying. Medium* 0·5 % *saponin containing* 5 % *alcohol. Tattersfield apparatus.*

Sample	Dry root mg./l.	No. of insects used	$B+M+D$ % allowing for controls	S.E.* % ±
D. malaccensis, Kinta (W. 211)	1147	45	100	—
	918	49	100	—
	734	51	98·0	2·0
	551	47	100	—
	367	51	76·0	8·6
	184	48	15·0	7·5
D. malaccensis, var. Sarawakensis (W. 212)	367	49	98·0	2·0
	275	51	84·0	3·9
	231	48	87·2	3·7
	184	48	46·8	13·4
	140	48	40·4	5·8
	91·8	49	2·1	—
D. elliptica, Changi (W. 214)	166	50	87·7	5·5
	148	52	76·4	8·6
	129	50	71·4	9·7
	111	49	52·1	9·0
	92·3	49	41·7	10·9
	73·8	50	22·4	12·0
Controls	—	97	2·1	

* Standard errors calculated on percentage mortalities before allowing for controls.

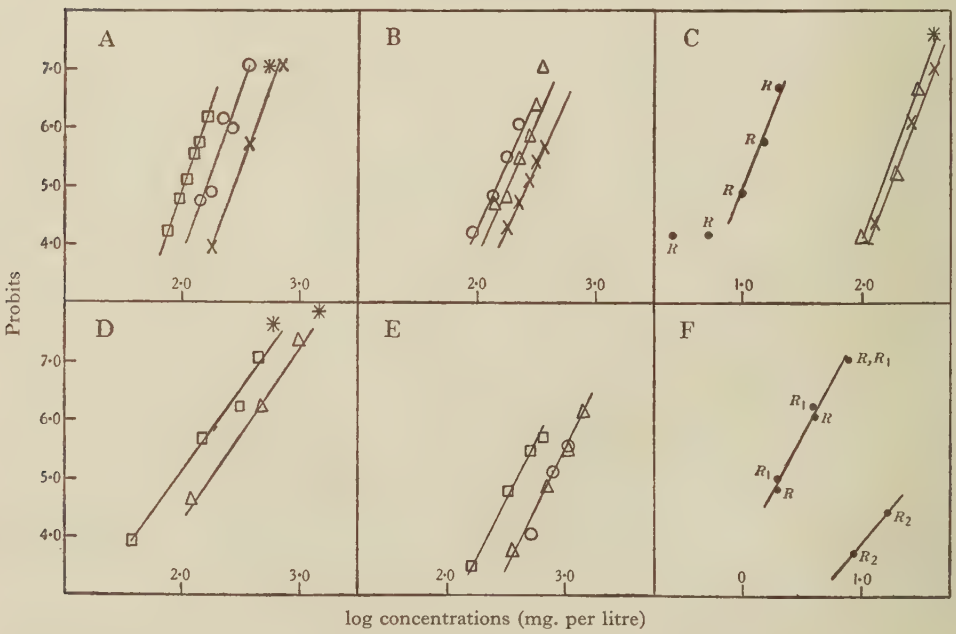

Fig. 1. Probits plotted against log concentrations of root, rotenone or inert Kinta-type resin.

× *D. malaccensis*, Kinta (W. 211) root.
○ *D. malaccensis*, Sarawakensis (W. 212) root.
△ *D. elliptica*, Sarawak creeping (W. 213) root.
□ *D. elliptica*, Changi (W. 214) root.

R Rotenone.
R_1 Rotenone + Kinta-type inert resin.
R_2 Kinta-type inert resin.
* Value for 100 % mortality.

TABLE 2. *Comparison of the toxicities of* D. malaccensis, *Kinta* (*W.* 211), D. malaccensis, *var. Sarawakensis* (*W.* 212) *and* D. elliptica, *Sarawak creeping* (*W.* 213) *roots, to* Aphis rumicis *from thistles. Tests of* 27 *July* 1938. *Fivefold replication of* 10 *insects. Results one day after spraying. Medium* 0·5 % *saponin containing* 5 % *alcohol. Tattersfield apparatus.*

Sample	Dry root mg./l.	No. of insects used	*B*+*M*+*D* % allowing for controls	S.E.* % ±
D. malaccensis, Kinta (W. 211)	367	49	74·5	2·4
	322	50	66·7	6·6
	276	50	54·2	8·1
	230	48	39·3	12·8
	184	47	24·7	3·9
	138	49	4·4	—
D. malaccensis, var. Sarawakensis (W. 212)	229	51	85·7	5·1
	183	50	68·8	3·2
	137	49	42·6	5·0
	91·7	49	21·4	9·2
	68·7	51	6·0	—
	45·8	49	10·7	6·1
D. elliptica, Sarawak creeping (W. 213)	369	50	97·9	2·0
	323	50	91·7	3·7
	277	51	79·6	7·8
	230	51	69·4	3·2
	184	48	41·4	9·2
	138	50	37·5	10·4
	92·2	49	2·2	—
	69·1	51	10·1	6·8
Controls	—	100	4·0	—

* Standard errors calculated on percentage mortalities before allowing for controls.

TABLE 3. *Comparison of the toxicities of rotenone*, D. malaccensis, *Kinta* (*W.* 211) *and* D. elliptica, *Sarawak creeping* (*W.* 213) *roots, to* Aphis rumicis *from broad beans. Tests of* 18 *July* 1939. *Fivefold replication of* 10 *insects. Results one day after spraying. Medium* 0·5 % *saponin containing* 10 % *alcohol. Tattersfield apparatus*

Sample	Rotenone mg./l.	Dry root mg./l.	No. of insects used	*B*+*M*+*D* % allowing for controls	S.E.* % ±
Rotenone†	20·2	—	47	95·6	4·0
	15·2	—	50	77·6	6·2
	10·1	—	47	45·7	8·7
	5·1	—	46	20·1	9·6
	2·5	—	51	20·0	5·0
D. malaccensis, Kinta (W. 211)	—	520	49	100	—
	—	390	45	97·8	2·4
	—	260	44	86·1	4·6
	—	130	52	27·3	6·7
	—	65	47	6·6	3·8
D. elliptica, Sarawak creeping (W. 213)	—	392	47	100	—
	—	294	41	95·0	6·6
	—	196	48	57·4	15·2
	—	97·9	47	19·7	4·0
	—	48·9	49	8·3	6·4
Controls (alcohol-saponin)	—	—	48	2·1	—

* Standard errors calculated on percentage mortalities before allowing for controls.
† Rotenone used was 99·8 % pure by rotation and 99·5 % pure by dichloracetic acid titration.

		mg. dry root/l. for 5 probits
Data of section A.	*D. malaccensis*, Kinta (W. 211)	281·8
	,, Sarawakensis (W. 212)	162·2
	D. elliptica, Changi (W. 214)	104·7
	W. 211 : W. 212 : W. 214 : : 1 : 1·74 : 2·69.	
Data of section B.	*D. malaccensis*, Kinta (W. 211)	263·0
	,, Sarawakensis (W. 212)	151·4
	D. elliptica, Sarawak creeping (W. 213)	182·0
	W. 211 : W. 212 : W. 213 : : 1 : 1·74 : 1·45.	
Data of section C.	Rotenone	9·77
	D. malaccensis, Kinta (W. 211)	166·0
	D. elliptica, Sarawak creeping (W. 213)	144·5
	W. 211 : W. 213 : rotenone : : 1 : 1·15 : 17·0.	

The data were analysed statistically by the methods given by Bliss (1935), Irwin (1937) and Cochran (appendix to Tattersfield & Martin, 1938). The results are given in Table 4.

The *D. malaccensis*, Sarawakensis (W. 212) root was compared with the *D. malaccensis*, Kinta (W. 211) root on two occasions, showing toxicities 1·64 and 1·84 times that of the Kinta-type root. The significance of the difference in toxicities of the W. 212 root relative to that of the W. 211 root was determined by the formula

$$t = \frac{M_1 - M_2}{\sqrt{({s_{M_1}}^2 + {s_{M_2}}^2)}} = 1{\cdot}01.$$

For $P = 0{\cdot}05$, and $n = 13$, $t = 2{\cdot}16$.

The difference in the toxicities of the *D. malaccensis*, Sarawakensis root, relative to that of the *D. malaccensis*, Kinta root, is therefore not a significant one.

The level of susceptibility of the insects throughout the experimental period changed materially, as is seen from the median lethal doses of the Kinta-type root, determined from the freehand probit-log concentration lines (this page). This is accounted for by the fact that the toxicity trials extended over a period greater than one year. The median lethal doses of the four roots have therefore been reduced to a strictly comparable basis.[1]

The weighted mean log L.D. 50 ($\bar{z}_1$) for root W. 211, tested in all three experiments, is given by

$$\bar{z}_1 = \frac{w_1 z_1 + w_1' z_1' + w_1'' z_1''}{w_1 + w_1' + w_1''},$$

with variance

$$V(\bar{z}_1) = \frac{1}{w_1 + w_1' + w_1''},$$

where z_1, z_1', z_1'' are the median lethal doses of W. 211, determined from the equation $y = a + b(x - \bar{x})$ for each experiment and w_1, w_1', w_1'' are the appropriate weighting values, i.e. the reciprocals of the variances of the z values.

The weighted mean of the log relative potencies of the *D. malaccensis* root (W. 212) and of the Kinta-type (W. 211) root is given by

$$\bar{M}_2 = \frac{w_2 M_2 + w_2' M_2'}{w_2 + w_2'},$$

where M_2 and M_2' are the log relative toxicities of the W. 212 and W. 211 roots in the two experiments in which they are compared and w_2, w_2' of the weights M_2, M_2'.

[1] For the purpose of obtaining an unbiased comparison between the four roots, it proved convenient to use here a slightly different definition of M from that generally accepted. Instead of making use of two probit lines constrained to be parallel, M was taken as the difference in the two values of log L.D. 50 obtained from the probit lines first calculated; since these lines never departed significantly from parallelism, the alteration in the value of M was always small.

TABLE 4. *Relative toxicities to* Aphis rumicis *of derris roots W. 211, W. 212, W. 213, W. 214 and rotenone by statistical analysis. Data of Tables 1, 2 and 3*

	Data of Table 1		
	W. 211		W. 212
χ^2a	1·605 ($n=3$)		7·510 ($n=3$)
χ^2b	—	0·231	
M	—	0·215	
Relative potencies (antilog M)	1·0	—	1·64
S.E. of M (s_M)		±0·028	
S.E. of relative potencies		±0·105	
	W. 212		W. 214
χ^2a	7·510 ($n=3$)	—	0·908 ($n=4$)
χ^2b	—	0·033	
M	—	0·200	
Relative potencies (antilog M)	1·0	—	1·59
S.E. of M (s_M)		±0·027	
S.E. of relative potencies		±0·099	
	Data of Table 2		
	W. 211		W. 212
χ^2a	0·029 ($n=3$)	—	0·783 ($n=2$)
χ^2b	—	0·027	
M	—	0·265	
Relative potencies (antilog M)	1·0	—	1·84
S.E. of M (s_M)		±0·041	
S.E. of relative potencies		±0·173	
	W. 212		W. 213
χ^2a	0·783 ($n=2$)		7·092 ($n=4$)
χ^2b	—	0·273	
M	—	0·094	
Relative potencies (antilog M)	1·24	—	1·0
S.E. of M (s_M)	—	±0·027	
S.E. of relative potencies	—	±0·076	
	Data of Table 3		
	W. 211		W. 213
χ^2a	0·142 ($n=2$)		5·306 ($n=2$)
χ^2b	—	0·364	
M	—	0·025	
Relative potencies (antilog M)	1·0	—	1·07
S.E. of M (s_M)		±0·035	
S.E. of relative potencies		±0·086	
	Rotenone		W. 213
χ^2a	5·535 ($n=3$)		5·306 ($n=2$)
χ^2b	—	2·337	
M	—	1·205	
Relative potencies (antilog M)	16·03	—	1·0
S.E. of M (s_M)	—	±0·039	
S.E. of relative potencies	—	±1·279	

The data throughout may be regarded as not heterogeneous (for $P=0·05$, when $n=2$, $\chi^2=5·991$, when $n=3$, $\chi^2=7·815$, and when $n=4$, $\chi^2=9·488$), nor do the pairs of probit-log concentration lines depart significantly from parallelism ($P=0·05$, $n=1$, $\chi^2=3·481$).

Then for an unbiased estimate of the mean log L.D. 50 for the W. 212 root, we may take

$$\bar{z}_2 = \bar{z}_1 + \bar{M}_2.$$

$\bar{z}_2$ must be written as a linear function of the original observations, since the variances of the two parts are not independent, from which $V(\bar{z}_2)$, the square of the S.E. of this estimate, is derived:

$$V(\bar{z}_2) = \frac{1}{w_2 + w_2'} - \frac{1}{w_1 + w_1' + w_1''}.$$

The values for root W. 213, $\bar{z}_3$ and $V(\bar{z}_3)$, may be similarly calculated.

For root W. 214, occurring once in the experiments,

$$\bar{z}_4 = \bar{z}_1 + \bar{M}_4,$$

with variance

$$V(\bar{z}_4) = \frac{1}{w_4} - \frac{1}{w_1 + w_1' + w_1''}.$$

The values of the estimated log L.D. 50 for the four roots, with their standard errors, were as follows:

	Estimate of mean log L.D. 50 ($\bar{z}$)	S.E. of $\bar{z}$
D. malaccensis, Kinta (W. 211)	2·3757	±0·0125
,, Sarawakensis (W. 212)	2·1306	±0·0172
D. elliptica, Sarawak creeping (W. 213)	2·2598	±0·0174
,, Changi (W. 214)	1·9509	±0·0251

The toxicities of the roots relative to that of the *D. malaccensis*, Kinta root, may now be directly compared. The concentrations of the dry root required for 50% kill of *Aphis rumicis* are: W. 211 237·5, W. 212 135·1, W. 213 181·9, and W. 214 89·3 mg./l. The toxicities of the roots relative to that of the Kinta-type root are as follows:

D. malaccensis, Kinta (W. 211): *D. malaccensis*, Sarawakensis (W. 212): *D. elliptica*, Sarawak creeping (W. 213): *D. elliptica*, Changi (W. 214) : : 1·0 : 1·76 : 1·31 : 2·66.

The *D. elliptica*, Sarawak creeping root, is more toxic than the Kinta-type root, but less toxic than the *D. malaccensis*, Sarawakensis root, while the *D. elliptica*, Changi root, shows the greatest toxicity.

Relative toxicities of the roots to a stored products insect, Oryzaephilus surinamensis L.

The biological trials described above and made in order to correlate toxicity with the results of chemical examination of the roots were carried out using one species of insect, *Aphis rumicis*. Before a chemical method of evaluation of derris can be widely adopted, however, it becomes a matter of some importance to know whether the relative toxicities of a number of roots to one particular insect will be the same when the roots are tested against other insects of different genera.

In order to determine this, preliminary toxicity trials, using three of the roots under examination, were carried out against the sawtoothed grain beetle, *Oryzaephilus surinamensis*. The insects were reared in a constant temperature and humidity room (at 27° C. and 70% R.H.) on sultanas and flaked maize. Spraying trials, using for each concentration a fivefold replication of twenty-five insects, were carried out in an apparatus devised by Dr C. Potter, a description of which will be published later. The apparatus is designed to give a uniform deposit over an area of approximately 70 cm.², and is suitable for use with quickly

moving insects. Trials were first carried out using the resins suspended in a medium of 0·5% sulphonated lorol containing 10% of alcohol. The deposit used was of the order of 1·2 mg./sq. cm. The insects after spraying were returned to the constant temperature room and examined 24 hr. later. The percentages of badly affected, moribund and dead insects were taken in assessing the results. These are given in Table 5 and the corresponding probit-log concentration lines in Fig. 1 D.

TABLE 5. *Comparison of the toxicities of* D. elliptica, *Sarawak creeping* (*W.* 213) *and* D. elliptica, *Changi* (*W.* 214) *roots to* Oryzaephilus surinamensis. *Tests of* 21 *June* 1939. *Fivefold replication of* 25 *insects. Results one day after spraying. Medium* 0·5% *sulphonated lorol containing* 10% *of alcohol. Potter spraying apparatus*

Sample	Dry root mg./l.	No. of insects used	$B+M+D$ % allowing for controls	S.E.† % ±
D. elliptica, Sarawak creeping (W. 213)	1480	142	100	—
	1000	127	99·1	0·8
	480	128	88·1	1·9
	120	126	35·3	7·9
D. elliptica, Changi (W. 214)	619	125	100	—
	458	117	98·0	2·0
	310	127	88·1	5·0
	149	51*	74·9	3·5
	37·1	132	13·7	4·2
Controls (alcohol-sulphonated lorol)	—	129	16·3	—

* Some insects lost.

† Standard errors calculated on percentage mortalities before allowing for controls.

The relative toxicities determined by statistical analysis are given in Table 6.

TABLE 6. *Relative toxicities to* O. surinamensis *of derris roots W.* 213 *and W.* 214 *by statistical analysis. Medium* 0·5% *sulphonated lorol containing* 10% *alcohol*

	W. 213		W. 214
$\chi^2 a$	1·377 ($n=2$)	—	4·714 ($n=3$)
$\chi^2 b$	—	0·073	
M	—	0·248	
Relative potencies (antilog M)	1·0	—	1·77
S.E. of M (s_M)	—	±0·042	
S.E. of relative potencies	—	±0·170	

Further trials were carried out using the resins of W. 212, W. 213 and W. 214 suspended in 0·5% saponin containing 10% alcohol. The deposits used were of the same order as those given with the sulphonated lorol-alcohol spray solutions. The results are given in Table 7, the relative toxicities determined by statistical analysis in Table 8, and the probit-log concentration lines in Fig. 1 E.

The data quoted may be regarded as not heterogeneous, while the pairs of probit-log concentration lines do not depart significantly from parallelism.

The *D. elliptica*, Changi (W. 214) root was compared in each experiment with the *D. elliptica*, Sarawak creeping (W. 213) root, showing toxicities 1·77 and 1·95 times that of the latter. This difference was not significant when tested by

$$t=\frac{M_1-M_2}{\sqrt{(s_{M_1}{}^2+s_{M_2}{}^2)}}=0{\cdot}799.$$

(For $P=0{\cdot}05$, $n=10$, $t=2{\cdot}228$).

TABLE 7. *Comparison of the toxicities of* D. malaccensis, *var. Sarawakensis* (*W.* 212), D. elliptica, *Sarawak creeping* (*W.* 213) *and* D. elliptica, *Changi* (*W.* 214) *roots to* Oryzaephilus surinamensis. *Tests of* 17 *August* 1939. *Fivefold replication of* 20 *insects. Results one day after spraying. Medium* 0·5% *saponin containing* 10% *alcohol. Potter spraying apparatus*

Sample	Dry root mg./l.	No. of insects used	$B+M+D$ % allowing for controls	S.E.* % ±
D. malaccensis,	1070	117	70·8	4·5
var. Sarawakensis (W. 212)	803	110	54·9	5·8
	535	104	16·5	8·8
D. elliptica,	1431	91	87·5	5·8
Sarawak creeping (W. 213)	1073	101	69·3	5·9
	716	109	44·1	6·6
	358	99	11·2	4·8
D. elliptica, Changi (W. 214)	668	88	74·1	3·7
	501	85	68·4	5·9
	334	98	42·0	5·5
	167	73	6·5	4·9
Controls (alcohol-saponin)	—	118	3·4	—

* Standard errors calculated on percentage mortalities before allowing for controls.

TABLE 8. *Relative toxicities to* O. surinamensis *of derris roots W.* 212, *W.* 213 *and W.* 214 *by statistical analysis. Medium* 0·5% *saponin containing* 10% *of alcohol*

	W. 212		W. 213
$\chi^2 a$	1·785 ($n=1$)	—	0·772 ($n=2$)
$\chi^2 b$	—	2·976	—
M	—	0·020	—
Relative potencies (antilog M)	1·0	—	1·05
S.E. of M (s_M)	—	±0·025	—
S.E. of relative potencies	—	±0·059	—

	W. 213		W. 214
$\chi^2 a$	0·772 ($n=2$)	—	1·092 ($n=1$)
$\chi^2 b$	—	1·375	
M	—	0·289	
Relative potencies (antilog M)	1·0	—	1·95
S.E. of M (s_M)	—	±0·030	
S.E. of relative potencies	—	±0·135	

To *Aphis rumicis* the *Derris elliptica*, Sarawak creeping (W. 213) root showed a toxicity of 0·74 times that of the *D. malaccensis*, Sarawakensis (W. 212) root, but in the one preliminary test against *Oryzaephilus surinamensis* its toxicity is not significantly different from that of the Sarawakensis root. Again the *Derris elliptica*, Changi (W. 214) root was approximately twice as toxic to *Aphis rumicis* as was the *Derris elliptica*, Sarawak creeping (W. 213) root, while to *Oryzaephilus surinamensis* its toxicity was 1·77 and 1·95 times greater than that of the W. 213 root.

From these preliminary experiments, there is no evidence to show that the relative toxicities of these roots to a stored product insect will differ materially from the values obtained using an aphid, although the levels of susceptibility of the two types of insect to the derris extracts in saponin-alcohol media have differed considerably.

In the experiments with *Oryzaephilus surinamensis* the toxicity of the *Derris elliptica*, Changi root, relative to that of the *D. elliptica*, Sarawak creeping root, tested in a sulphonated

lorol-alcohol medium, was not significantly different from the relative toxicity determined in a saponin-alcohol medium. In the two experiments, however, there was a wide divergence in the concentrations of root required to give 50% mortality of the insects, although the experimental technique and the amounts of deposit used remained constant. In the trials using the sulphonated lorol medium, the median lethal dose of the *D. elliptica*, Changi root, was 93 mg./l., while in the saponin medium the median lethal dose was 379 mg./l. An interval of two months had elapsed between the experiments, but it was not thought that a change in the level of susceptibility of the insects alone could account for the wide difference in the concentrations of root required for 50% mortality. Further trials were therefore carried out using the *D. malaccensis*, Sarawakensis root, in which its toxicity in a sulphonated lorol-alcohol medium was directly compared with its toxicity in a saponin-alcohol medium. The previous results were confirmed; the toxicity of the root in the sulphonated lorol medium was appreciably greater than its toxicity in the saponin medium.

It is clear that the effect of medium upon toxicity is an important consideration in the utilization of rotenone-containing plants.

The effect of the biologically inert fraction of derris resin

Derris resin contains something of the order of 10% of inert material, believed to possess emulsifying properties. It is possible that this inert fraction, while possessing little or no toxicity itself, enhances the toxic action of the active principles by a purely physical effect. Toxicity trials have indicated that this does not take place to any extent. The inert fraction was separated from the *D. malaccensis*, Kinta root, by a single extraction of the resin in ether solution with 2% aqueous potash. The deeply coloured alkaline extract was washed with ether and the resin recovered by acidification and re-extraction with ether. A red-brown brittle material resulted which may have contained small amounts of toxicarol.

Tests against *Macrosiphoniella sanborni* (Gill.) (the chrysanthemum aphis), reared in a cool glasshouse, were then carried out using pure rotenone in 0·5% saponin solution containing 10% of alcohol and the same concentrations of rotenone with the addition of 25% by weight of the Kinta-type inert resin. The resin showed only a slight toxic effect when tested at four times the concentrations used in admixture with the rotenone. No significant difference in position was seen between the lines relating the probit values to the log concentrations of rotenone tested separately and mixed with the resin (Fig. 1 F).

Chemical investigations

Fractionation of the D. elliptica, *Changi resin, with the determination of the specific rotations of the fractions in benzene*

The root (350 g.) was extracted with chloroform, the resin dissolved in benzene, and the specific rotation was determined. The benzene solution was extracted four times with 5% potash, washed, and dried over anhydrous sodium sulphate. The specific rotation was again determined. The benzene was removed finally by warming under reduced pressure and the resin dissolved in carbon tetrachloride. The rotenone-carbon tetrachloride complex was separated, the mother liquor concentrated, pure rotenone (10 g.) added and a second crystallization carried out. 16·87 g. of complex resulted, of which the purity, determined by trituration with alcohol saturated with rotenone, was 89·7%. The additional rotenone separated by the second crystallization amounted therefore to 0·88 g.

The solvent was removed from the mother liquor, the resin dissolved in ether and extracted twice with 2% and five times with 5% potash. The ether solution was washed, dried and concentrated, and

the resin precipitated three times by the addition of an excess of petroleum ether. The ether-petroleum ether-soluble fraction was obtained as a viscous oil. The mixed solvent was removed from the precipitated resin under reduced pressure to yield a yellow powder, the "deguelin concentrate".

The specific rotations of the fractions, with the concentrations used in their determination, are given in Table 9.

TABLE 9. *Specific rotations in benzene of fractions of the* D. elliptica, *Changi resin*

	Concentration %	$[\alpha]_D^{20°}$
Original resin	3·86	−121°
Resin after potash extraction of benzene solution	2·76	−140°
Resin after removal of rotenone	3·28	−68°
Ether-petroleum ether-soluble oil	3·54	−46°
"Deguelin concentrate"	2·00	−82°

The rotations of mixtures of the active principles

An attempt was made to determine to what extent the rotations of mixed active principles behaved additively. Solutions in benzene were made containing known amounts of rotenone, the toxicarol precursor and the "deguelin concentrate" preparations, and their rotations determined in a 10 cm. tube at 20° C.

The rotations of the preparations of the active principles used were:

	Concentration %	$\alpha_D^{20°}$	$[\alpha]_D^{20°}$
Rotenone	4·00	−9·04	−226°
	1·80	−4·14	−230°
Toxicarol precursor (contained 10 % sumatrol)	4·00	−3·20	−80·0°
	1·67	−1·38	−82·6°
"Deguelin concentrate"	3·99	−3·19	−79·9°
	2·00	−1·63	−81·7°

The observed rotations of solutions in benzene of the mixed active principles are given below. From graphs showing the changes in rotation of the preparations of the active principles with concentration, the rotations in circular degrees that should be given by the following concentrations of each constituent were calculated.

	Concentration %				Observed		Calculated	
	Rotenone	Toxicarol precursor	"Deguelin concentrate"	Total	$\alpha_D^{20°}$	$[\alpha]_D^{20°}$	$\alpha_D^{20°}$	$[\alpha]_D^{20°}$
Solution 1	0·79	2·41	0·80	4·00	−4·28°	−106·9°	−4·47°	−111·8°
Solution 2	1·58	0·81	1·60	3·99	−5·39°	−134·8°	−5·64°	−141·3°

The rotations of the active principles would therefore appear to be additive in the mixture, the discrepancy, if real, amounting to less than 5 %.

Quantitative fractionation of the samples used in the biological trials, with the determination of the specific rotations of the fractions in benzene and acetone

The roots were extracted by percolation with ethyl acetate for 3 hr., the solvent was removed, and the resins, in ether solution, extracted once with 2 % and twice with 5 % potash. The ether- and potash-soluble fractions were recovered, the latter by acidification with dilute hydrochloric acid and extraction with ether, and weighed. The ether-soluble resin was taken up with carbon tetrachloride, the complex separated, and the residual resin recovered. In all cases, the solvents were removed by heating under reduced pressure at a temperature below 60° C. until the resins were of constant weight. The rotations of all the fractions were determined in both benzene and acetone. The results are given in Table 10.

The influence of the toxicarol upon the rotation is seen when the rotations in benzene and acetone of the *D. malaccensis* resins and toxicarol-containing fractions are compared. The potash-soluble resin shows a laevo-rotation in benzene and a dextro-rotation in acetone.

TABLE 10. *Fractionation of derris resins with the determination of the specific rotations of the fractions in benzene and acetone*

	% of dry root	$[\alpha]_D^{20°}$ Benzene	$[\alpha]_D^{20°}$ Acetone
D. malaccensis, Kinta W. 211			
Total resin	25·1	−66·3° (4·05)	+30·5° (4·04)
Neutral resin	7·3	−87·2° (3·01)	−28·1° (3·01)
Potash soluble resin	13·2	−53·8° (3·05)	+43·3° (3·05)
Rotenone calculated from complex	1·11	—	—
Rotenone calculated from % purity of complex (91·2)	1·01	—	—
Deguelin concentrate	6·4	−64·6° (3·09)	−18·6° (3·04)
D. malaccensis, var. Sarawakensis, W. 212			
Total resin	20·3	−97·1° (4·01)	−14·6° (4·01)
Neutral resin	13·1	−124·6° (3·01)	−48·9° (3·02)
Potash soluble resin	5·1	−39·2° (2·99)	+27·8° (1·51)
Rotenone calculated from complex	3·61	—	—
Rotenone calculated from % purity of complex (90·8)	3·28	—	—
Deguelin concentrate	10·1	−86·4° (3·02)	−27·7° (3·01)
D. elliptica, Sarawak creeping, W. 213			
Total resin	13·6	−95·3° (4·03)	−26·1° (4·01)
Neutral resin	11·6	−109·0° (3·00)	−35·4° (3·00)
Potash soluble resin	1·3	*	*
Rotenone calculated from complex	3·67	—	—
Rotenone calculated from % purity of complex (92·8)	3·41	—	—
Deguelin concentrate	7·8	−62·9° (3·00)	−4·4° (3·00)
D. elliptica, Changi, W. 214			
Total resin	22·1	−121·5° (4·09)	−42·8° (4·07)
Neutral resin	19·7	−132·8° (3·01)	−50·5° (3·02)
Potash soluble resin	1·2	*	*
Rotenone calculated from complex	7·89	—	—
Rotenone calculated from % purity of complex (94·4)	7·44	—	—
Deguelin concentrate	11·9	−75·5° (3·01)	−14·4° (3·02)

Percentage concentrations of resins at which rotations were determined are given in brackets.
* Too dark to read in polarimeter.

Correlation between the rotation values and the biological activities of the roots and their resins

(*a*) *Application to derris roots.* The roots under examination showed the following relative toxicities to *Aphis rumicis*, determined biologically (p. 282):

D. malaccensis, Kinta : *D. malaccensis*, Sarawakensis : *D. elliptica*, Sarawak creeping : *D. elliptica*, Changi : : 1·0 : 1·76 : 1·31 : 2·66.

The resin contents of the roots (ethyl acetate extraction) were as follows (see Table 12):

	% of dry root
D. malaccensis, Kinta	25·3
,, Sarawakensis	20·8
D. elliptica, Sarawak creeping	14·1
,, Changi	22·7

In assessing the toxic values of the roots by rotational methods, it is necessary to take into account these varying percentages of total extractives. This may be done by extracting a definite weight of

root, the resin being dissolved and made up to a definite volume with determination of the rotation. Assume 20 g. of each air-dried root were extracted (ethyl acetate) and the resin was dissolved in benzene and made up to 100 ml. The rotations in circular degrees of the extracts of the four roots, with the concentrations of resins used, would be:

	Concentration of resin %	$\alpha_D^{20°}$
D. malaccensis, Kinta	4·64	−3·08°
,, Sarawakensis	3·79	−3·68°
D. elliptica, Sarawak creeping	2·56	−2·44°
,, Changi	4·17	−5·07°

It is known that the specific rotations of the active principles change slightly with concentration, so that a strictly valid assessment of toxic value by comparing these rotation figures cannot be made in view of the differing concentrations of resin used. Neglecting this concentration factor, the ratios of the rotations, taking the Kinta-type root as the standard of comparison, are respectively as

1·0 : 1·19 : 0·79 : 1·65.

On this basis of assessment the roots are not even placed in their correct order of biological activity, the *D. elliptica*, Sarawak creeping root, appearing to be less effective than the Kinta-type root, and the *D. malaccensis*, Sarawakensis, and *D. elliptica*, Changi roots, being considerably undervalued with respect to the Kinta-type root.

(*b*) *Application to derris resins.* The possibility of determining the relative toxicities of the resins of derris root by comparing their specific rotations has to be examined. By calculation from the relative toxicities of the four roots determined biologically and the percentages of material extracted by ethyl acetate, the relative toxicities of the four resins are as follows:

D. malaccensis, Kinta (W. 211) : *D. malaccensis*, Sarawakensis (W. 212) : *D. elliptica*, Sarawak creeping (W. 213) : *D. elliptica*, Changi (W. 214) : : 1·0 : 2·14 : 2·35 : 2·96.

The specific rotations of the resins at 4 % concentration in benzene (data of Table 10) were as follows:

	$[\alpha]_D^{20°}$
D. malaccensis, Kinta	−66·3°
,, Sarawakensis	−97·1°
D. elliptica, Sarawak creeping	−95·3°
,, Changi	−121·5°

These values are in the ratios of 1·0 : 1·46 : 1·44 : 1·83.

The toxicities of the W. 212, W. 213, and W. 214 resins are definitely undervalued with respect to the Kinta-type resin.

Rotational methods cannot be regarded as entirely satisfactory for the assessment of the biological activity of all types of either derris root or derris resin. The discrepancies in the values are to be explained by the varying composition of the resins from different types of root, coupled with the fact that there appears to be no direct correlation between the toxicities of the active principles and their rotatory powers in benzene.

Determination of the benzene extracts of the roots with the application of the method of evaluation of Jones & Smith (1936)

This method of evaluation has been applied to the roots under consideration. The benzene extractives were determined by Soxhlet extraction of 5 g. of root for 20 hr., the extracts being dried in an oven at 100° C. for ½ hr. periods until the loss of weight between successive weighings was less than 5 mg. The values were:

	% of dry root
D. malaccensis, Kinta (W. 211)	23·8
,, Sarawakensis (W. 212)	19·3
D. elliptica, Sarawak creeping (W. 213)	12·7
,, Changi (W. 214)	20·9

When, as an approximation, the purified rotenone contents (see Table 12) were added to one-half of the benzene extract figures minus the rotenone, the relative toxicities of the roots were indicated as follows:

	Rotenone + 0·5 (rotenone-free resin) % of dry root
D. malaccensis, Kinta (W. 211)	12·6
„ Sarawakensis (W. 212)	11·7
D. elliptica, Sarawak creeping (W. 213)	8·1
„ Changi (W. 214)	14·4

From these values, the W. 212 and W. 213 roots appear to be less toxic than the Kinta-type (W. 211) root, and the Changi (W. 214) root appears only slightly more toxic than the Kinta root. To *Aphis rumicis* the W. 212 root was 1·76 times as toxic, and the W. 214 root was 2·66 times as toxic as the W. 211 root.

This method of assessment, devised for use with houseflies, does not hold for the evaluation of the toxicities of extreme types of derris to aphides. The failure of the method may be, to some extent, explained by the different type of insect used. The rotenone-free resin, however, will vary in composition, particularly with regard to the content of toxicarol, according to the species of derris employed. In view of the differing toxicities of the active principles to aphides it is difficult to conceive that the rotenone-free resins from different species of root will show equal toxicities to this type of insect. A more detailed specification of the non-rotenone fraction of the resin would appear to be necessary before its true toxic value can be assessed.

Fractionation of the resins with the determination of their "rotenone equivalents"

We have shown (Martin & Tattersfield, 1936) that rotenone would separate readily as the carbon tetrachloride complex from a solution of the resin of the *D. malaccensis*, Sumatra-type root, after removal of the toxicarol and other alkali-soluble material from an ether solution by means of potash. The potash fractionation of ether solutions of derris resins provides a rapid qualitative test for distinguishing chemically between the *D. elliptica* and *D. malaccensis* roots, and has proved a valuable preliminary step in much of our work. The adoption of the method led directly to the first separation of the naturally occurring toxicarol; this was obtained, in minute crystalline form, from the ether solution of the resin recovered from the insoluble potassium salt separated from a Sumatra-type resin. The optically active toxicarol was recrystallized from ethyl acetate. It was later obtained by direct crystallization from a Sumatra-type resin in ethyl acetate without seeding. The fractionation method has been used as a preliminary step to later work (Harper, 1939) resulting in the separation of *l*-elliptone.

There has been the risk, visualized from the first, of degradation due to the action of the alkali. If, however, the extraction by alkali is quickly carried out, no degradative changes appear to ensue. A minor modification has been incorporated to render the method applicable to roots rich in rotenone. A mixture of ether and benzene containing 25 % of benzene by volume is used as solvent. This prevents crystallization of the rotenone from resins rich in this active principle and assists in reducing the vapour pressure of the ether.

The fractionation method has been quantitatively applied to the four roots under examination. Sufficient root to give approximately 5 g. of resin was extracted by percolation with ethyl acetate for 3 hr. The apparatus[1] used consisted of a glass liner fitting into a wider glass tube connected with a 250 ml. flask and condenser. The powdered root is supported in the liner on a pad of cotton wool, and rapid extraction at the boiling point of the solvent is effected. Experiments showed that ethyl acetate, ethylene dichloride and chloroform were equally effective in extracting the toxic principles with a minimum of extraneous matter. Solvent efficiencies of a similar order have been found by Jones & Sullivan (1938). The percentages of resin extracted by percolation of 25 g. portions of the *D. elliptica*, Changi (W. 214) root, with different solvents are given in Table 11.

The hot percolation method, using ethyl acetate, ethylene dichloride or chloroform is rapid, and has the advantages over the method of Worsley (1936) of being automatic and requiring little more than 150 ml. of solvent. The extraction by percolation of finely divided derris powders may be assisted by the admixture of previously extracted coarser material.

The solvent (ethyl acetate) was removed by distillation followed by heating on the water bath under reduced pressure for 15 min., and the resin weighed. It was dissolved in 100 ml. of benzene-ether, and transferred with further small quantities of solvent to a 500 ml. *pear*-shaped separating funnel. The

[1] Quickfit and Quartz Ltd., London, W. 1, catalogue nos. EX 4/10, EX 4/15, CX 3/05, extraction type flask.

TABLE 11. *Percentages of total extract from the* D. elliptica, W. 214 *root, by hot percolation with different solvents*

	% of dry root	
	First 2 hr.	Second 2 hr.
Ethyl alcohol	32·0 (22·0*)	1·1
Acetone	24·4 (22·3*)	0·7
Ethyl acetate	22·2	0·3
Ethylene dichloride	21·7	0·2
Chloroform	21·0	0·5
Trichlorethylene	20·7	0·6
Benzene	19·4	0·7
Carbon tetrachloride	10·9	0·4
Petroleum ether	4·0	2·0

* Soluble in benzene.

solution was extracted by *gentle rotation*, with 50 ml. of 2 % potash, the alkaline layer being removed as quickly as possible and added to 40 ml. of benzene-ether in a second separating funnel. Two further rapid extractions, as before, with 5 % potash were carried out and an excess of water immediately added to the main benzene-ether solution to reduce the concentration of any residual potash. The combined alkaline extracts were washed with the 40 ml. of benzene-ether, which was added to the main solution. A second washing, with 40 ml. of benzene-ether, was carried out, and the combined benzene-ether solutions washed three times with water, allowing effective drainage between each. The benzene-ether solution was run through a layer of anhydrous sodium sulphate, the solvent removed by distillation followed by warming under reduced pressure and the resin weighed.

The "neutral resin" was dissolved in five times its weight, in ml., of carbon tetrachloride saturated with rotenone at 0° C. (2·72 g./l.) and placed in the refrigerator overnight. The complex was separated, washed with a minimum quantity of cold carbon tetrachloride saturated with rotenone (approximately 10 ml.) allowed to dry at room temperature overnight, and weighed. All apparatus was precooled before filtration.

Determination of the purity of the complex

This was carried out by a polarimetric method. The crystals were removed from the crucible, well mixed and a 4 % solution in benzene prepared. The rotation was determined at 20° C. using a 10 cm. water-jacketed tube.

The specific rotation of the pure complex in benzene was found to vary from $-168°$ at 2 % concentration to $-166°$ at 4 % concentration. The percentage purity of a complex is therefore given by $100\alpha/6{\cdot}64$, where α is the observed rotation in circular degrees of a 4 % solution at 20° C. using a 10 cm. polarimetric tube.

This method involves the assumption that the impurities present in the complex show no optical rotation. Actually, they are likely to show a specific rotation approximately equal to one-quarter that of rotenone. The error involved in the determination of the percentage purity of a complex obtained after potash extraction of the original resin, however, is not large, and the rotation due to the adsorbed resin-like impurities may in any case be allowed for if this measure is considered necessary. The values obtained have, in general, agreed well with those obtained using the dichloracetic acid titration method of Jones (1938).

When a solution of complex in benzene other than of 4 % concentration was used, the percentage purity was determined by reading, from the graph showing the change in the specific rotation of the complex with concentration, the specific rotation at the concentration used, with calculation of the theoretical angle of rotation for this concentration.

The percentage of the "toxicarol fraction" which has been removed by the potash may be determined from the difference in the percentages of the original and "neutral" resins, while the percentage of "neutral resin" minus the percentage of purified rotenone has given an assessment of the deguelin fraction, expressed as the "deguelin concentrate".

The results of the alkaline fractionation of the roots under examination are given in Table 12.

We have shown (Tattersfield & Martin, 1938) that the rotenone- and toxicarol-free resins obtained from the Sumatra-type derris (*D. malaccensis*) and *D. elliptica* were of the order of

TABLE 12. *Fractionation of derris resins W.* 211, 212, 213, 214

	% of dry root			
	D. malaccensis, Kinta (W. 211)	*D. malaccensis*, Sarawakensis (W. 212)	*D. elliptica*, Sarawak creeping (W. 213)	*D. elliptica*, Changi (W. 214)
Total extract = A	25·3	20·8	14·1	22·7
Neutral resin = B	10·1	16·5	11·7	20·1
Rotenone from complex	1·68	4·73	3·73	8·51
% purity of complex by rotation	87·2	87·6	92·8	93·3
Purified rotenone = R	1·46	4·14	3·46	7·94
Toxicarol fraction* $A-B=T$	15·2	4·3	2·4	2·6
Deguelin concentrate fraction $B-R=D$	8·6	12·4	8·2	12·2

* In addition to toxicarol this fraction may contain sumatrol, malaccol and other material such as dispersing agents.

one-fifth as toxic as rotenone, while the toxicarol precursor and sumatrol, removed in the fractionation method by the potash, were of the order of $\frac{1}{13}-\frac{1}{15}$ as toxic as rotenone. More knowledge is required upon the detailed constitution of the "deguelin concentrate" fraction from various types of root. Elliptone, if present in the root, will be included in the deguelin concentrate on fractionation of the resin, and shows, if our preliminary work is confirmed, a toxicity of the same order as that of the deguelin concentrate from a *D. elliptica* root. Moreover, its presence will not invalidate the determination of rotenone, since it is not precipitated as a complex with carbon tetrachloride.

It is therefore possible, from the amounts and relative toxicities of the fractions, to calculate for each root a percentage "rotenone equivalent" value, determined by the summation of the percentage of rotenone, $\frac{1}{5}$ of the percentage of the "deguelin concentrate" fraction and $\frac{1}{15}$ of the percentage of the toxicarol fraction, i.e.

$$\text{rotenone equivalent, R.E.} = R + D/5 + T/15.$$

The values for the "rotenone equivalents" of the four roots were as follows:

	"Rotenone equivalent"
D. malaccensis, Kinta (W. 211)	4·19
,, Sarawakensis (W. 212)	6·91
D. elliptica, Sarawak creeping (W. 213)	5·26
,, Changi (W. 214)	10·55

These values are in the ratios of 1 : 1·65 : 1·26 : 2·52.

The relative toxicities of the roots to *Aphis rumicis*, were respectively (see p. 282):

1 : 1·76 : 1·31 : 2·66.

A reasonably close correlation is seen to exist between the ratios of the rotenone equivalents and the relative toxicities of the roots determined biologically.

DISCUSSION

The derris extracts of commerce depend for their complete insecticidal action upon the presence of varying amounts of rotenone, the most toxic constituent, and other active principles of widely differing toxicities. A number of methods of chemical evaluation of the root have been suggested, in which allowances for the additional toxicity due to the non-rotenone fraction of the extract have been made.

The method of Jones & Smith (1936), in which a figure expressing the toxic value of the root is obtained by adding to the rotenone content a definite percentage of the non-rotenone fraction of the extract according to the solvent used, has been found to be inadequate when applied to the different species of roots examined. The use of the method implies that the rotenone-free resins from different types of root will have equal toxic values, but in view of the varying amounts of the different active principles in derris extracts from different species of root, this is not the case. The constituent which shows the greatest variation in content in derris resins is the toxicarol precursor. While only approximately one-fifteenth as toxic as rotenone to *Aphis rumicis*, it may comprise 60% of the extract in the case of a Kinta or Sumatra-type (*Derris malaccensis*) root, but is present only in small quantities, if at all, in the extract of a *D. elliptica* root. It is possible that the method of Jones & Smith (1936) is satisfactory for the evaluation of roots of one species, *D. elliptica*, of which the extracts do not, in general, show wide variations in the relative contents of the active principles.

The evaluation of derris roots of different species by methods based upon optical rotation values is unsound in view of the absence of a direct correlation between the optical rotatory powers of the active principles and their toxicities. Again, the use of an optical rotation method may provide an assessment of roots of a given species, but such roots may be evaluated amongst themselves by rotenone, ether extract or methoxyl determinations (Tattersfield & Martin, 1935).

It was in an attempt to allow for the varying toxic values of the rotenone-free resins from different species and varieties of root that the method of determination of the "rotenone equivalent", based upon the toxicities of the deguelin and toxicarol fractions relative to that of rotenone, was applied. Extreme types of roots, of authenticated species and of known origin, and ranging from the low-rotenone, Kinta-type variety of *D. malaccensis* to the rich *D. elliptica*, Changi root, were taken to test this method of assessing their relative toxicities, with the expectation that the more normal roots widely encountered in commerce would then also be amenable to satisfactory evaluation. The method of evaluation by determination of the "rotenone equivalent" would appear to give a reasonable assessment of the toxicities of these roots. It is therefore put forward as a step towards the solution of the approximate evaluation of derris. Further work is needed to determine the relative toxicities of the three fractions concerned in the determination of the rotenone equivalent when tested against representatives of orders of insects other than those already used.

In theory, the maximum rotenone equivalent percentage value that a root could show at present is of the order of 30, assuming it contained 30% of extract consisting entirely of rotenone, while the minimum possible value is 2·0, given by a root containing 30% of extract, all of which passes into the alkaline layer on fractionation.

The method of approximate assessment consisting of adding to the percentage of rotenone one-fifth of the percentage of the "deguelin concentrate" and one-fifteenth of the percentage of the toxicarol fraction, may be applied to derris resins, the rotenone equivalent being then expressed as a percentage of the resin.

The compositions of the resins of the roots examined were:

	% of resin			
	W. 211	W. 212	W. 213	W. 214
Purified rotenone	5·9	19·7	24·8	34·8
Deguelin concentrate	34·0	59·6	58·2	53·7
Toxicarol fraction	60·1	20·7	17·0	11·5

The values of the rotenone equivalents of the resins are therefore as follows: W. 211 16·7, W. 212 33·0, W. 213 37·6 and W. 214 46·3.

These are in the ratios respectively of 1·0 : 1·98 : 2·25 : 2·77.

The ratios of the toxicities of the resins to *Aphis rumicis* calculated from the determined relative toxicities of the roots and the percentages of material extracted from each root by ethyl acetate were as follows (see p. 288): 1·0 : 2·14 : 2·35 : 2·96.

From its rotenone equivalent value the resin of the *D. elliptica*, Changi root, should show a toxicity equal to a little less than one-half that of pure rotenone.

While the inert fraction of a derris resin does not seem to enhance the toxic effect of rotenone, and presumably, of the other active principles, there may be a synergistic action between the active principles themselves. Bliss (1939) has detected, by statistical means, evidence of such synergism between the active principles of derris, using data published by ourselves. The present data were considered inadequate for a valid test for synergistic action to be made.

If the basis for the assessment of the toxicities of the roots relative to that of rotenone (determination of rotenone equivalent) is valid, rotenone should show a toxicity greater than that of a given root in inverse proportion to the percentage rotenone equivalent of the root. Thus the rotenone equivalent of the *D. elliptica*, Sarawak creeping (W. 213) root, was 5·26%, so that pure rotenone should be 100/5·26 = 19·0 times more toxic than this root. Actually by toxicity trials using *Aphis rumicis* rotenone was 16·0 times more toxic than the root (Table 4).

This lower relative toxicity, if significant, may be due to failure to observe the full toxic action of the rotenone, or what is more likely from the work of Bliss (1939), to the fact that the root is showing a slightly greater toxicity than that predicted from the contents and toxicities of its individual active principles. In other words, the discrepancy between the observed and calculated relative toxicities of rotenone to root may furnish indirect evidence of some synergistic action between the active principles, but not sufficient to invalidate the assessment of the root by the determination of its rotenone equivalent.

The alkaline fractionation method enables one to ascertain in derris root the content of purified rotenone as well as the rotenone equivalent. At present, however, it is not suggested that such a procedure should supplant existing methods for the determination of this active principle. The method is put forward here in the hope that it may be fairly tried out and its utility as a means of evaluating derris fully explored.

Summary

1. The relative toxicities to *Aphis rumicis* of different species and varieties of derris root have been determined. A *Derris elliptica*, Changi root, was two and one-half times, a *D. malaccensis*, Sarawakensis root, was one and three-quarter times, and a *D. elliptica*, Sarawak creeping root, was one and one-quarter times as toxic as a *D. malaccensis*, Kinta root.

2. Preliminary trials showed that the relative toxicities of three of the roots to a stored products insect, *Oryzaephilus surinamensis*, were of a similar order. All toxicity data have been subjected to statistical analysis.

3. Various methods suggested for the chemical evaluation of derris have been examined and discussed. Methods based upon optical rotation values have been shown to fail in the evaluation of roots and resins of different species, while the method of Jones & Smith (1936),

in which a definite toxic value is given to the non-rotenone fraction of the derris extract, has been found to be inadequate when applied to the roots under consideration.

4. The determination of the percentage "rotenone equivalent" values of the roots, based upon the alkaline fractionation of the resins and the toxicities of the deguelin and toxicarol fractions relative to that of rotenone, has given a reasonably close estimate of the toxicities of the derris roots examined. The application of the method to the assessment of the toxic value of derris resins is described.

I wish to express my indebtedness to Drs Tattersfield and C. Potter for assistance in the biological trials, to Dr Tattersfield for criticism and advice, to Dr Potter for the supplies of *Oryzaephilus surinamensis*, and to Mr D. J. Finney of the Statistical Department, Rothamsted Experimental Station, for valuable assistance in the statistical examination of the toxicity results. I am indebted to the Ministry of Agriculture and to the Colonial Development Fund for grants which enabled this work to be carried out.

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THE MAINTENANCE OF HIGH ATMOSPHERIC HUMIDITIES FOR ENTOMOLOGICAL WORK WITH GLYCEROL-WATER MIXTURES

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(With 2 Text-figures)

THE control of atmospheric humidity in closed spaces for entomological work is usually accomplished by keeping aqueous solutions of sulphuric acid or of inorganic salts inside the experimental chamber (Buxton, 1931; Buxton & Mellanby, 1934). If large numbers of insects are to be kept or a frequent inspection made closed chambers should be avoided, since insects tend to build up high humidities around themselves and, if the chamber is often opened, the required humidity may never be attained uniformly within it.

In such cases a stream of air at the required humidity is sometimes passed through the experimental chamber after bubbling through Woulff's bottles filled with small pieces of pumice stone and the appropriate mixture of sulphuric acid and water. It has been the practice to use desiccators as experimental chambers and to keep the insects in tubes, open (but for a voile cap) at one end only, and packed upright inside the desiccator. There is much dead space in such a chamber and even with a rapid stream of air at 10–15 l./hr. I have found that 3 or 4 hr. may elapse before the required humidity is attained after reclosing the chamber.

Moreover, dilute sulphuric acid requires careful handling; and constant attention is necessary in eliminating the acid spray and in testing the specific gravity of rather small quantities of acid from the Woulff's bottles. If the strength of the acid is tested by weighing the water vapour in the air which passes through it, considerable time may be spent on the operation. The action of the acid on foreign particles carried into it or on the perforated zinc used to support tubes inside the chamber produce fumes which perhaps may be toxic to insects.

These disadvantages have, to some extent, been eliminated from the apparatus described below.

The experimental chamber. The chamber is a straight sided battery or museum jar with internal dimensions of about $7 \times 4 \times 2\frac{1}{2}$ in. On each narrow side a hole 1 cm. in diameter is bored. On one side the hole is near the bottom and on the other side near the top of the jar. A glass lead-in tube projects through the hole near the bottom and is bent so as to direct an air stream on to the floor of the jar. Inside the jar at the bottom are two glass rods, each with two right angle bends, which lean one within the other on opposite sides of the jar. These rods support a sheet of perforated zinc on which the tubes containing the insects rest (Fig. 1). The lid of the jar is a piece of ground glass with the edges smeared with vaseline; it fits to the ground edges at the top of the jar.

Tubes for the insects. I have used the apparatus for keeping bed-bugs. These were confined, singly or in pairs, in bottomless glass tubes, 2×1 in., with voile caps on each end. These tubes were packed horizontally into the battery jar with their ends towards the wider

sides, leaving a space of about $\frac{1}{4}$ in. between the ends of the tubes and the sides of the jar. The air stream thus flowed up the sides from the bottom of the jar and past the bugs as they sat on the voile. Even if the dead space, the amount of which is minimized, takes some time to reach the required humidity after the jar has been closed, the insects themselves are likely to come most quickly into contact with the correct humidity from the air which flows past them.

Humidity control. A mixture of glycerol and water has been used to maintain relative humidities above 85%. It is probably impracticable to control humidities lower than 75% R.H. with glycerol-water mixtures since the viscosity of the solutions is high and air is not easily bubbled through them. Also, their specific gravity changes very quickly.

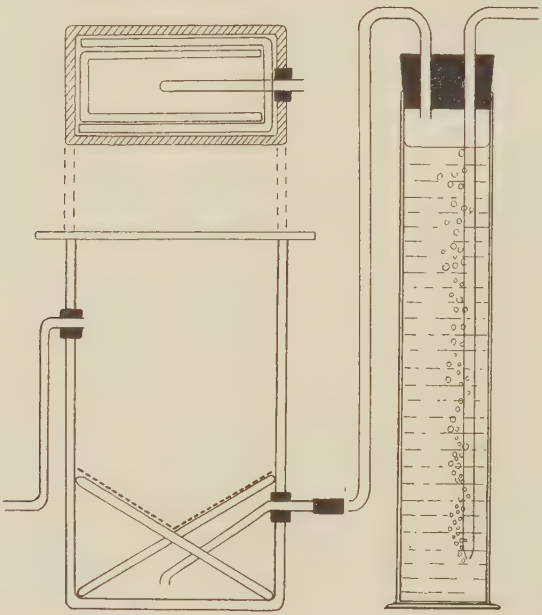

Fig. 1. Battery jar for holding insects and cylinder of glycerol-water mixture for humidifying air.

About 80 c.c. of the glycerol-water mixture is poured into a 100 c.c. measuring cylinder; a bung with two glass tubes, as shown in Fig. 1, is fixed into the open end of the cylinder and the outlet tube is connected to the battery jar. Air is bubbled through the glycerol at a rate of about 10 l./hr. thus allowing for a complete change of air within the jar several times in 1 hr. The specific gravity of the mixture is tested every 3 or 4 days by taking the bung out of the cylinder and dropping a hydrometer into the glycerol. Water is then added until the necessary density is regained.

The whole procedure is remarkably quick and simple and without the inconveniences associated with the use of sulphuric acid; it takes about 2 min. compared with perhaps 1 hr. if acid is used and the atmosphere tested by weighings with a Landseidle bulb. A constant watch can be kept on the specific gravity of the glycerol mixture and no harmful fumes are likely to be produced. Only one cylinder is necessary and pumice stone need not be used if

the inlet jet is small. The glycerol mixture, unlike the solutions of some salts and potassium hydroxide, gives very similar relative humidities over a wide temperature range (Table 1). The apparatus could, of course, be used with salt solutions instead of glycerol. The battery jars can be connected in series and will fit compactly into a small incubator.

Table 1 gives determinations I have made with a Landseidle bulb at various temperatures for glycerol mixtures giving relative humidities mainly above 80%. Table 2 and Fig. 2 give relative humidities from 0 to 100% and the corresponding density of the solutions: this table

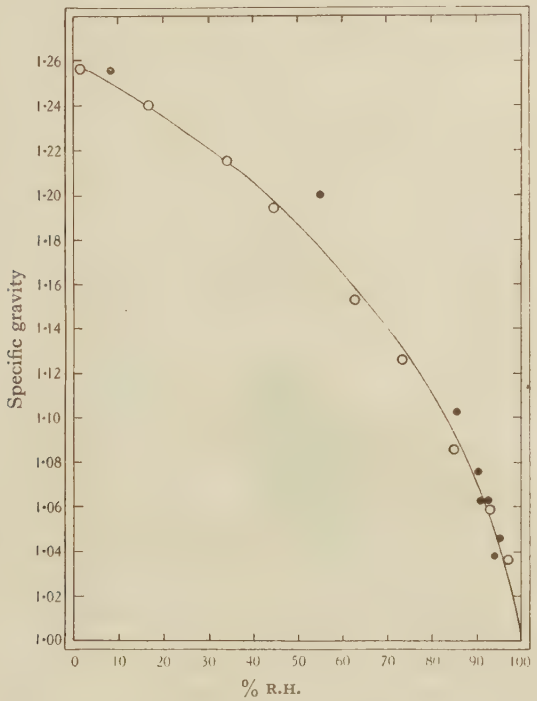

Fig. 2. Specific gravity and the corresponding relative humidities for glycerol-water mixtures.

○ Data from Landolt-Börnstein. Sp.gr. at 20° C.; R.H. at 25° C. (Table 2).
● Data from Landseidle bulb determinations. Temperature, 21–23° C. (Table 1).

is compiled from Landoldt-Bornstein. Table 3 contains a typical series of consecutive changes in the density of a mixture while in use (giving about 90% R.H.) with the corresponding approximate variation in relative humidity. To keep the relative humidity at about 90%, 2 or 3 c.c. of water must be added every 3 or 4 days; the variation in relative humidity is then no more than ±2% at constant temperatures between 7° and 23° C. if the air is fairly moist before it is bubbled at about 10 l./hr. through the mixture.

I was concerned only with the control of humidities near 90% R.H. and the determinations in Table 1 were sufficient for my purpose. It is desirable that more accurate observations should be made for various concentrations of glycerol and water at temperatures between 0

and 30° C. for the method is a very promising one where high humidities must be controlled for long periods with very little attention.

TABLE 1. *Relative humidity of air, determined with a Landseidle bulb, after bubbling through a cylinder of glycerol-water mixture of various concentrations at about 10 l./hr.*

Mixture	Temp. of mixture C.	Sp.gr.	R.H. %. Successive determinations	Mean R.H. %
I	23·0	1·038	93·8	
	22·0–22·5		97·2, 95·0, 92·1, 91·6, 94·7	
II	21·5	1·046	92·1, 91·5, 98·0, 94·8, 94·8, 95·3, 98·0, 93·5	94·8
	13·5	1·049	96·9, 97·3, 96·5, 95·0	96·4
III	23·0	1·063	91·9, 91·9, 92·9, 92·4	92·3
	22·0–22·3		91·6, 91·7, 91·2	91·5
	21·5		92·9	
IV	22·0–22·3		88·3, 88·3	
	21·8	1·076	88·4, 91·3, 91·3	
V	21·5–21·8	1·102	86·2, 86·2, 82·4	85·6
VI	. 7·8–8·4	1·070	89·5, 87·4, 87·4, 88·7	88·3
VII	21·0–23·0	1·200	54·4, 55·4, 56·7, 55·5, 53·5, 53·6	54·9
VIII	22·5–23·0	1·256	8·6, 8·8, 8·6	8·7

Specific gravity changes with temperature

Sp.gr. at 23·3° C. = 1·076 Sp.gr. at 21·5° C. = 1·046
„ „ 8·0° C. = 1·079 „ „ 13·5° C. = 1·049

TABLE 2. *Compilation from Landolt-Börnstein. Vapour pressures of glycerol-water solutions of various percentages at 25° C.* (5 *Auflage*, 1931: *Zweiter Teil, p.* 1328). *Specific gravities of solutions of various strengths are mean values at 20° C.* (5 *Auflage*, 1923: *Erster Band, p.* 458)

% glycerol solution	Water V.P. mm.	Sp.gr.	% R.H.
0	23·7	0·998	100·0
15·5	23·0	1·036	97·0
25·0	22·0	1·059	92·8
35·0	20·2	1·085	85·2
50·0	17·4	1·126	73·4
60·0	14·8	1·153	62·4
75·0	10·5	1·194	44·3
83·0	8·0	1·215	33·8
92·0	4·0	1·240	16·9
98·0	0·4	1·256	1·7
100·0	—	1·261	0

TABLE 3. *Records of seven consecutive inspections, selected at random, of the sp.gr. of a glycerol-water mixture with air bubbling through it at about* 10 *l./hr. at* 23° *C.*

Interval between inspections days	Sp.gr. after the addition of water	Sp.gr. at end of interval	Approx. variation in % R.H.
3	1·073	1·080	90–88
4	1·075	1·088	89–86
2	1·073	1·083	90–87
4	1·070	1·087	90–86
3	1·074	1·084	89–87
3	1·073	1·081	90–88
4	1·074	1·082	89–87

SUMMARY

A method is described in which glycerol-water mixtures are used to maintain high atmospheric humidities (above 85% R.H.) for long periods with a minimum of attention. The method has been used with insects. A convenient container is described in which the amount of dead space is minimized and in which the humidity required in the neighbourhood of the insects tends to be quickly regained after the apparatus has been closed.

My best thanks are due to Prof. P. A. Buxton for his help and hospitality, and to the Medical Research Council for the grant which I have received.

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REVIEWS

Propagation of Horticultural Plants. By GUY W. ADRIANCE and FRED. R. BRISON. Pp. viii+314, 182 figs. London: McGraw Hill Publishing Co., Ltd. 1939. 20*s.* 0*d.*

This book is intended to serve as a text-book for basic courses in horticulture and related courses in American Agricultural Colleges. The first three chapters explain in a concise manner the structure and functions of plants, special attention being given to the practical aspects of pollination and fertilization. The rest of the book describes the methods practised in American nurseries and gardens for the propagation of plants by seeds and by the various asexual methods, viz. by bulbs and related structures, layers, cuttings and grafts. The methods of transplanting and the growing and handling of nursery stock are also considered. The specific practices followed in the propagation of certain useful and ornamental plants are described in detail. The plants thus treated include the cultivated tree and bush fruits of British gardens, also roses and conifers, and in addition, typical American economic plants such as Pecan, Persimmon, Citrus, Avocado, Blueberry and Tung Nut. There is a useful chapter on the relation of propagation practices to diseases. Throughout the book the descriptions are clear and concise and are excellently illustrated, and numerous references are given to current research work. Although primarily concerned with American horticultural practices this volume is of interest and value to British horticulturists and horticultural students. It would be of more value if botanical names of the plants described were used more consistently, as American popular plant names, e.g. Tung Nut and Blueberry, have little or no meaning for British readers. The terms "graftage", "layerage", "cuttage" are unfamiliar, and perhaps irritating, to British horticulturists, but these are minor criticisms of a work of real practical value.

ADELA G. ERITH.

Diseases of Bulbs. By W. C. MOORE. Pp. vi+176, 58 figs. Ministry of Agriculture and Fisheries. Bull. No. 117. London: H.M. Stationery Office. 1939. 4*s.* 0*d.* net.

Mr Moore's "bulbs" are all included in the families Liliaceae (Hyacinth, Tulip, Lily, with brief notes on *Bulbocodium*, *Chionodoxa*, *Colchicum*, *Convallaria*, *Fritillaria*, *Muscari*, *Ornithogalum*, and *Scilla*), Amaryllidaceae (*Narcissus* and Snowdrop), and Iridaceae (*Gladiolus*, *Iris*, *Crocus*, with brief notes on *Freesia*, *Ixia*, *Montbretia*, and *Tigridia*). Under each host the diseases are grouped according to their etiology (bacteria, fungi, virus, eelworm, non-parasitic, miscellaneous minor troubles), and where possible, each disease is discussed under the following headings: brief introduction, history and geographical distribution, nature and description of the disease, nomenclature and characters of the parasite, host range and varietal susceptibility, and control in the "bulb" and in the field. The author's aims were "to provide the grower with an account in popular language of the symptoms, spread and control of each of the common diseases, and to furnish the plant pathologist and research worker with a guide to the literature on the more uncommon diseases and on the technical aspects of the subjects". The book concludes with a bibliography of over 700 citations referred to in the text; it is illustrated by a series of plates containing 58 excellent and, with three exceptions, original photographs; there is a brief contents but no Index; and it is attractively bound in stiff board covers.

The book is to a large extent based on compilation but the data have been analysed and marshalled by a severely critical mind and the work incorporates and is illuminated by fourteen years of first hand experience. Although there is much in the work which is open to discussion I find nothing which is open to criticism. It is already evident that by the time a second edition is called for some present obscurities will have been clarified, and that certain problems, e.g. the relationships and nomenclature of the *Sclerotinia-Botrytis* group, may need considerable revision, but at the time of publication the author would not have been justified in accepting any other position than that he has adopted. At a price of four shillings this book is almost the best value for money of any plant disease publication I know and the Ministry of Agriculture deserve all praise for making work of this quality so easily available.

W. B. BRIERLEY.

Destructive and Useful Insects: their habits and control. By C. L. METCALF and W. P. FLINT. 2nd Edition. Pp. xvi+981, 584 figs. and 17 tables. London: McGraw-Hill Publishing Co. Ltd. 1939. £2. 10*s*.

This is an important addition to the literature of economic entomology by the Professor of Entomology at the University of Illinois and the Chief Entomologist at the Illinois Agricultural Experiment Station. It is a large and heavy and somewhat expensive volume, but nothing less could contain a comprehensive account of the injurious and useful insects, mites and spiders, of the whole of the United States of America and Southern Canada, in addition to a general introduction to entomology and insect control. Though a second edition, it is to all intents and purposes a new book. The first edition, which appeared in 1928, has been enlarged and almost wholly rewritten, as a result of the "sweeping changes that have taken place in entomological practices" since that date.

The first ten chapters—some 330 pages—are devoted to the general aspects of the subject and form a valuable introductory course in economic entomology. The matter dealt with includes the external morphology, internal anatomy, physiology and development of insects, the place of insects in the animal kingdom and the orders of insects, together with a useful summary of methods of insect control and an account of apparatus for applying insecticides. There is also an instructive general discussion of the ways in which insects are injurious or valuable to man, and in Tables III and IV an attempt is made to draw up a debit and credit account between insects and the American people. The grand total economic loss in the United States in 1936 is estimated at the imposing figure of 1,326,442,567 dollars, but against this there appears on the credit side, in addition to the money value of insect products, a long list of items, including the services of insects as pollinators, as food for other animals, in controlling destructive insects, in scientific investigations and in medicine and surgery, the value of which, however, the authors do not venture to estimate in dollars.

The second section of the book consists of ten chapters each dealing with the insects injurious to one particular group of crops, for example vegetable crops, deciduous fruit crops, citrus crops; and finally there are three chapters on household and stored product pests, insects injurious to domestic animals, and insects attacking or annoying man. The wide range of climatic conditions in the great area covered, with the correspondingly wide range of crops and pests, supplies material for a thorough introduction to applied entomology.

Special features of the book are the system of cross references adopted in the second part and the abundant use of keys and tables throughout. Since many insects are pests of more than one group of crops, treatment under the heading of crops, though the most convenient classification to adopt, tends to some repetition. This is to a great extent avoided by a simple system of cross referencing, easy to use, which enables one quickly to turn up all the information given about any particular species. In addition, there is a full index.

The authors have been to great trouble to construct a large number of keys and tables. There are field keys for the identification of pests injuring each of the important crop plants and for pests of stored products and of domestic animals and man, the divisions being based primarily on the nature of the injury caused. In addition, keys are given to the orders of insects both in the adult and immature stages, the latter being of special interest, and there are synopses of the various orders. Much of the information given about insecticides and other subjects is also summarized in tabular form. The successful use of keys is somewhat of a personal matter and it is rather difficult to assess their value, but it is evident that they have been very carefully drawn up and no doubt the authors' long experience of teaching and field work has shown their usefulness.

The book is written throughout in simple language, easy to read, and is conspicuously free from misprints. Illustrations are very numerous, though not all of equal value and some have a rather antiquated appearance; many are reproduced from Bulletins of the State Agricultural Experiment Stations. References to important American publications are given at the end of each section.

As a compendium of information on the applied entomology of North America, this fine text-book contains much of interest for English students and practising entomologists who will find it of great value as a reference book. It should have a place on the shelves of all our biological libraries.

C. T. GIMINGHAM.

REPORT OF THE COUNCIL OF THE ASSOCIATION OF APPLIED BIOLOGISTS FOR THE YEAR 1939

THE Officers and Council of the Association were as follows:

President: C. T. GIMINGHAM, B.Sc., F.I.C.

Vice-Presidents: H. W. MILES, D.Sc., H. WORMALD, D.Sc.

Hon. Treasurer: J. HENDERSON SMITH, M.B., Ch.B.

Hon. Secretaries: General and Botanical: W. P. K. FINDLAY, M.Sc.; Zoological: H. F. BARNES, M.A., Ph.D.

Hon. Editors of Annals of Applied Biology: General and Botanical: Prof. W. B. BRIERLEY, D.Sc.; Zoological: C. T. GIMINGHAM, B.Sc., F.I.C.

Other Members of the Council: G. E. BLACKMAN, M.A., H. A. DADE, A.R.C.S., W. J. DOWSON, M.A., D.Sc., S. D. GARRETT, M.A., H. G. H. KEARNS, Ph.D., H. W. MILES, D.Sc., W. C. MOORE, M.A., H. C. F. NEWTON, B.Sc., F. R. PETHERBRIDGE, M.A., A. ROEBUCK, N.D.A., E. R. SPEYER, M.A., H. WORMALD, D.Sc.

The Association has met on three occasions during the year. Three other meetings had been fixed for the Autumn but, after the outbreak of the war, the Council considered that it was inadvisable to proceed with the arrangements for these. For the first time for many years a provincial meeting was held; by kind invitation of the authorities of Manchester University the Summer meeting was held there on 8–10 June. The interesting programme of lectures and visits which had been arranged by Dr Miles was much appreciated by all members attending the meeting.

The attendance at the London meetings was on the average 38 Members and 25 visitors. Twenty-two Members and 11 visitors attended the meeting in Manchester.

Fourteen new Members were elected during the year and 5 Members resigned. The Council has to record with regret the deaths of Prof. Carpenter, who was President of the Association from 1910 to 1913 and of Mr M. A. Bailey and Mr E. H. Richards who had been Members since 1914 and 1921 respectively.

The Association now numbers 336 Members including 12 Honorary Members; an increase of 6 over the previous year. Of the ordinary Members, as far as is known 273 are resident in the British Isles and 53 in the Empire or in foreign countries.

The following papers were brought before the Association during 1939:

10 February. *Discussion on Sugar Beet Problems.*

W. MORLEY DAVIES, M.A., B.Sc.: Acidity and manganese deficiency problems in connexion with sugar beet growing.

A. W. GREENHILL, Ph.D., M.Sc., F.I.C.: The effect of boron on the growth and quality of sugar beet.

F. R. PETHERBRIDGE, M.A.: Sugar beet pests.

A. ROEBUCK, N.D.A.: Pests of the sugar beet crop in the Midlands.

H. H. STIRRUP, M.Sc.: Sugar beet diseases.

17 March. *Discussion on Testing and Diseases of Seeds.*

C. C. BRETT, M.A.: The production, handling, testing and diseases of seeds.

R. W. G. DENNIS, Ph.D.: Notes on seed transmission of *Phoma lingam* in relation to dry rot of swedes in Scotland.

MARY NOBLE, Ph.D.: Notes on *Pullularia pullulans* in ryegrass seed and seed-testing methods as affecting detection of certain seed-borne diseases.

V. McM. Davey, Ph.D.: Hybridization in Brassicae and the occasional contamination of seed stocks.

W. A. R. Dillon-Weston, M.A.: Modern methods of seed disinfection.

9 June. Summer Meeting. *Symposium on Applied Biology and Crop Production.*

Prof. G. W. Robinson, M.A.: Pedology, the study of the soil.

G. H. Bates, D.Sc.: Botany and ecology in relation to crop production.

H. W. Thompson, M.Sc.: Agricultural entomology in Yorkshire.

F. T. Bennett, Ph.D., D.Sc.: Some past and present crop disease problems in the north of England.

During the meeting at Manchester visits were made to the seed trial grounds of Messrs Garton's and to the University of Manchester's Entomological Field Station where the wireworm investigations were examined. The party also visited the Seedling Raising Station of the Forestry Commission in Delamere Forest, a fruit plantation, and a model dairy.

The Association again had the privilege of holding meetings in the Botanical Department and Metallurgical Lecture Theatre of the Imperial College of Science and Technology, and the Council takes this opportunity of expressing its grateful thanks, on behalf of the Association, for this hospitality.

The Hon. Editors of the *Annals of Applied Biology* report that in 1939 the volume comprised pp. xii + 849 and 43 plates, as against pp. xii + 891 and 37 plates for 1938, this slight reduction being in accordance with a ruling of Council. Including papers published as "Proceedings", vol. **26** contained 60 papers and 45 reviews, as against 61 papers and 41 reviews for vol. **25**. Of the papers in the 1939 volume, 34 were by Members of the Association and 26 by non-members. The papers may roughly be classified as follows: general applied botany 13; mycology and fungal diseases 17; bacterial diseases 1; viruses and virus diseases 6; general applied zoology 2; entomology and insect pests 10; plant protection 4; helminthology and nematode diseases 2; applied microbiology 3; general 2.

The several parts of the *Annals of Applied Biology* were published on the following dates: Part 1, 16 February; Part 2, 25 May; Part 3, 25 August; Part 4, 28 November.

W. P. K. FINDLAY
H. F. BARNES } *Hon. Secretaries*

REPORT OF THE HON. TREASURER FOR THE YEAR ENDING 31 DECEMBER 1939

During the year ending 31 December 1939 subscriptions and entrance fees, including arrears paid in, amounted to £261. 4*s*. 6*d*., a decline of £63. 8*s*. 6*d*. as compared with 1938. Income from the sale of the current volume of the *Annals of Applied Biology* and from reprints amounted to £786. 18*s*. 9*d*., an increase of £23. 15*s*. 9*d*., mostly due to larger sales of the volume. The size of the *Annals* amounted to 850 pages as against 891 in the previous year, and the cost of printing to £1248. 19*s*. 5*d*., a reduction of £7. In the whole year there has been an excess of expenditure over income of £112. 1*s*. 7*d*.

The Association has an excess of assets over liabilities of £650. 9*s*. 0*d*. To this sum £177 is contributed by the estimated value of the stock of volumes of the *Annals*. This is an asset which is not quickly realizable and would drop very greatly in value if publication of the *Annals* ceased, and the estimate is put at a low figure. In actual fact sale of back volumes has in the last three years brought in over £300 and our assets are larger than appears from the balance sheet. They have been intentionally reduced by about £100 during each of the last few years, but in view of the present increased costs of production and the uncertainty as to the future it will be advisable for us to keep our expenses more nearly within our income. It will help greatly to that end if members will pay their subscriptions promptly and lose no chance of obtaining new members.

J. HENDERSON SMITH
Hon. Treasurer

THE ASSOCIATION OF APPLIED BIOLOGISTS

Dr. *ANNALS OF APPLIED BIOLOGY*, INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1939 *Cr.*

Expenditure	£ s. d.	£ s. d.	Income	£ s. d.	£ s. d.
To Estimated Value of Stock, 1 January 1939		150 16 0	By Sales—Current Volume		661 16 0
To Cambridge University Press		1248 19 5	By Sales—Back Volumes, Parts and Sets		76 18 10
To Copies bought in		18 9	By Sales of Reprints		126 2 9
			By Advertisements		1 3 0
			By Estimated Value of Stock, 31 December 1939		177 16 0
			By Balance, carried down		356 17 7
		£1400 14 2			£1400 14 2

Dr. GENERAL INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1939 *Cr.*

Expenditure	£ s. d.	£ s. d.	Income	£ s. d.	£ s. d.
To *Annals of Applied Biology*, balance brought down		356 17 7	By Members' Subscriptions		251 5 0
To Sundry Out-of-Pocket Expenses of Secretaries, Editors and Treasurer		32 13 10	By Entrance Fees		9 19 6
To Audit Fee		4 4 0	By Interest on National Savings Certificates and Bank Deposit		20 9 4
			By Balance, being Excess of Expenditure over Income for the year		112 1 7
		£393 15 5			£393 15 5

BALANCE SHEET, 31 December 1939

Liabilities and Surplus	£ s. d.	£ s. d.	Assets	£ s. d.	£ s. d.
Sundry Creditors:			Cash at Bank:		
Cambridge University Press	386 15 1		Current Account	103 8 10	
Audit Fee Reserve	4 4 0		Deposit Account	1 0 0	
Sundry Expenses	14 12 0				104 8 10
		405 11 1	Debtors for Subscriptions in arrear and considered good		110 17 0
Subscriptions paid in advance		3 15 0	401 National Savings Certificates		666 13 3
Excess of Assets over Liabilities:			Stock of *Annals of Applied Biology* at estimated value		177 16 0
As Balance Sheet of 31 December 1938	762 10 7				
Less: Balance of Income and Expenditure Account for 1939	112 1 7				
		650 9 0			
		£1059 15 1			£1059 15 1

J. HENDERSON SMITH, *Hon. Treasurer*

We certify that the foregoing Accounts are properly drawn up in accordance with the books, vouchers and documents produced to us, and, in our opinion, the Balance Sheet exhibits a true and correct view of the state of the affairs of the Association according to such books, vouchers and documents.

(Signed)
H. J. COX & CO.
Incorporated Accountants
Auditors

HARPENDEN, 11 *March* 1940